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THE EFFECT OF CHOLINE SUPPLEMENTATION ON PHYSICAL AND MENTAL PERFORMANCE OF ELITE RANGERS

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14. Abstract <p>Dietary availability of choline, the precursor of the neurotransmitter, acetylcholine, is sufficient to provide the body's requirements under normal conditions. However, some reports indicated plasma choline levels fall following certain types of strenuous exercise and that depletion of choline may limit performance, while oral supplementation may delay fatigue. A double-blind crossover design was used to determine the relationship between plasma choline and fatigue during and after a 4 hr strenuous exercise. Fourteen Army Rangers participated in this study (ages 19-33 years, mean body fat 11% and $\dot{V}O_{max}$ 60.3 $ml \cdot kg^{-1} \cdot min^{-1}$). Thirty mins after drinking a nonnutritive beverage with or without choline citrate (8.425 g), Rangers walked on a treadmill at 5.6 km/h, 3% grade, wearing a 29.5 kg rucksack for 20 km, equivalent to approximately 1950 Kcal energy expenditure. An identical dose of the choline supplement was given midway through the treadmill walk. Post-test run time-to-exhaustion, squat test, perceived exertion, marksmanship, short-term memory, mood states, lactate, glucose, CPK, lipids, and plasma choline were measured. Choline levels increased 128% after the run-to-exhaustion during the choline supplemented phase but remained stable under the placebo conditions. No significant effects were seen with choline supplementation on any outcome performance measures. Consequently, plasma choline was not depleted as a result of a weighted road march, a typical Ranger performance task, nor did the Rangers benefit from choline supplementation to enhance or delay fatigue under this exhaustive military task.</p>					
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ABSTRACT

Dietary availability of choline, the precursor of the neurotransmitter, acetylcholine, is sufficient to provide the body's requirements under normal conditions. However, some reports indicated plasma choline levels fall following certain types of strenuous exercise and that depletion of choline may limit performance, while oral supplementation may delay fatigue. A double-blind crossover design was used to determine the relationship between plasma choline and fatigue during and after a 4 hr strenuous exercise. Fourteen Army Rangers participated in this study (ages 19-33 years, mean body fat 11% and $\dot{V}O_2\text{max}$ 60.3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Thirty mins after drinking a nonnutritive beverage with or without choline citrate (8.425 g), Rangers walked on a treadmill at 5.6 km/h, 3% grade, wearing a 29.5 kg rucksack for 20 km, equivalent to approximately 1950 Kcal energy expenditure. An identical dose of the choline supplement was given midway through the treadmill walk. Post-test run time-to-exhaustion, squat test, perceived exertion, marksmanship, short-term memory, mood states, lactate, glucose, CPK, lipids, and plasma choline were measured. Choline levels increased 128% after the run-to-exhaustion during the choline supplemented phase, but remained stable under the placebo conditions. No significant effects were seen with choline supplementation on any outcome performance measures. Consequently, plasma choline was not depleted as a result of a weighted road march, a typical Ranger performance task, nor did the Rangers benefit from choline supplementation to enhance or delay fatigue under this exhaustive military task.

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LIST OF ABBREVIATIONS & ACRONYMS

ALICE	all-purpose, lightweight, individual carrying equipment
ANOVA	analysis of variance
BTPS	body temperature pressure saturated with water
CBC	complete blood count
DCM	distance center of mass of the target
DEXA	dual-energy X-ray absorptiometry
DMST	delayed-match-to-sample test
EKG	electrocardiogram
FDA	Food and Drug Administration
SusD	Sustainability Directorate
GRAS	generally regarded as safe
HDEV	horizontal deviation of shot
HDL	high density lipoproteins
HSGT	horizontal shot group tightness
LED	light emitting diode
LSRO	Life Science Research Office
MOS	military occupational specialty
NRD & EC	Natick Research, Development, & Engineering Center
PERC	Performance Enhancing Ration Component
POMS	Profile of Mood States
PT	physical training
RPE	rating of perceived exertion
SD	standard deviation
SE	standard error of the mean
SGT	shot group tightness
SOF	Special Operations Forces
STIME	sighting time (marksmanship)
USAMRMC	U.S. Army Medical Research & Materiel Command
USARIEM	U.S. Army Research Institute of Environmental Medicine
VDEV	vertical deviation of shot
VE	minute ventilation
VLDL	very low density lipoproteins

VO ₂ max	maximum oxygen uptake
VSGT	vertical shot group tightness

Units of Measurements:

cm	centimeter
g	gram
kg	kilogram
km	kilometer
L	liter
m	meter
mg	milligram
mL	milliliter
mmol	millimole
N	Newton
W	watt
μmol/L	micromole per L
mmol/L	millimole per liter

EXECUTIVE SUMMARY

The Performance Enhancing Ration Components (PERC) project supports the Science and Technology Objective "Nutritional Strategies to Enhance Soldier Performance." The focus of the PERC project is to demonstrate, through nutrition intervention, enhancement of physical and/or mental performance in sustained high-intensity work. In this PERC study, we examined the effects of a liquid form of choline supplement on physical and mental performance of Ranger soldiers.

On each of 2 test days, which were separated by 1 week of recovery, 14 Rangers performed a sustained 4-hr load carriage exercise with a 29.6 kg rucksack, walking at 5.6 km/h at a 3% grade. After walking for approximately 20 km, the subjects then performed a treadmill run-to-exhaustion, a marksmanship test, and a 45.5 kg squat test. Dietary intake of choline was controlled by having subjects restrict sources of choline from their diet for two days prior to each experimental trial. Dietary food records were used to assure compliance. Subjects were given a 16 oz test drink consisting of a base sweetened with aspartame supplemented with or without choline citrate (8.43 g). Two hours and fifty minutes after onset of exercise, subjects received another 16 oz test beverage.

We assessed whether choline supplementation affects performance and also whether there is a preferential effect based on the ability of specific types of muscular work. The exercise measures used to study physical performance included a run-to-exhaustion for aerobic endurance and a 45.5 kg squat test for muscular strength and endurance.

The total energy cost of the load carriage exercise was calculated as the hourly oxygen consumption multiplied by 5 kcals per liter of oxygen consumed, resulting in approximately 1950 calories expended.

The mean plasma choline levels under the placebo condition fell by only 1.5%. The mean plasma choline levels in the treatment condition rose by 128%. There was no indication that more prolonged walking would have resulted in depletion of plasma choline, nor is it likely that elevated levels of plasma choline through supplementation

are able to sustain strength or endurance performance. Additionally, there is no reason to believe that elevated plasma choline levels would impede performance.

The results of this study demonstrate that the neurophysiological response of runners and cyclists are potentially different from those of prolonged submaximal load carriage marchers. This suggests that any nutritional supplement that is reported to have ergogenic properties under a specific exercise condition may not always translate to other types of activities with different physiological and metabolic motor unit involvement.

CHAPTER ONE

BACKGROUND

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INTRODUCTION

This study was the first USARIEM/NRD&EC effort to investigate the nutrient choline for possible beneficial effects on physical and mental performance. Special Operation Forces (SOF) and combat units are trained to operate effectively while their bodies and minds are subjected to prolonged periods of combat stress. Choline is a naturally occurring nutrient that theoretically could have a unique potential effect on brain function and physical performance in its role as a precursor for the neurotransmitter acetylcholine. There have been only a few investigations into the ergogenic benefits of its use.

Choline's primary benefit appears to be its potential to improve endurance and reduce fatigue. U.S. combat doctrine emphasizes the need for 24-hr fighting capabilities. Special Operation Forces and combat units are truly unique from their counterparts, the elite athletes, to whom they are sometimes compared. Special Operation Forces' sustained work may take them well beyond the more brief and highly intense sporting competition. Combat fighters' sustained work/sustained operations

involve mental and physical precision that, if compromised, can have serious consequences resulting in diminished performance capacity.

Throughout the history of warfare, the combat soldier has been required to carry more weight than practical when deployed into battle (Marshall, 1950). Some of the most recent conflicts depicting combat soldier mobility were the Falklands and Grenada campaigns where soldiers carried heavy loads (in excess of 45 kg) for considerable distances. The inability to carry heavy loads contributed significantly to the high number of heat casualties and also to general fatigue which led to either poor fighting or no fighting at all.

Soldier mobility is defined as the ability of the soldier to move by foot with accompanying gear about the battlefield. Therefore, soldiers must rely primarily on their own personal mobility to carry the bulk of equipment and supplies. Current doctrine states that light infantry division soldiers will carry supplies and equipment to be self-sustained for a period of 48 hrs, after which resupply will be required by external combat service support. The U.S. Army Infantry Board has set 72 lbs as the desired weight limit for the approach march load. The 10th Special Forces Group (A) Certification Standard requires an annual 20 km rucksack march with an approximate 29.6 kg load within 4 hrs to measure a soldier's endurance. Prolonged load-bearing marches are an appropriate military relevant endurance task upon which to evaluate physical stress.

The primary dietary sources of choline are limited among the current components that make up our operational ration menus. If prolonged military work/tasks cause a significant reduction in plasma choline, and there is inadequate dietary replenishment, this could cause a potential decrease in the war fighters' capabilities in a sustained operational environment. Optimal nutritional status is strongly associated with maximal physical and mental performance; it is critical for ongoing scientific investigation into nutritional strategies that will target potential performance enhancing nutrients such as choline.

FUNCTIONS OF CHOLINE

Choline is a component of phosphatidylcholine (lecithin). It is important in the structure of all cell membranes, plasma lipoproteins, and pulmonary surfactants (Zeisel, 1981). It is a precursor for the biosynthesis of the neurotransmitter acetylcholine.

Human Requirements for Choline

The current dietary allowances for maintenance of good health published by the National Research Council in the Recommended Dietary Allowances, 10th Edition (1989), lists choline as a nutrient essential for some higher animals, but not for normal humans: thus, no specific dietary requirements have been issued.

Choline has been considered a dispensable nutrient for humans because of an endogenous pathway for its biosynthesis via the sequential methylation of phosphatidylethanolamine (Zeisel and Blusztajn, 1994). The human infant consumes a choline-rich diet. Breast milk contains approximately 1.5 mmol/L choline and choline esters. Choline is routinely added to commercially available infant formulas (approximately 1 mmol/L free choline per liter).

Free choline concentrations in blood serum vary closely with dietary consumption of choline and lecithin, a choline-containing phospholipid, with peak serum levels in humans occurring within several hours of ingestion (Cohen and Wurtman, 1976; Ziesel, Growden, Wurtman et al., 1980; Ziesel, DaCosta, Franklin et al., 1991). In the absence of disease, and under sedentary conditions, it is likely that choline concentration does not limit the synthesis of acetylcholine, a key central and peripheral neurotransmitter. Normal plasma choline concentrations for males can be expected to be in the range of 9.6 to 10.9 $\mu\text{mol/L}$ (Ziesel, DaCosta, Franklin et al., 1991).

Choline Markers

Blood choline concentrations decrease significantly during choline deficiency (Sheard, Tayek, Bistrian et al., 1986; Burt, Hanin, and Brennan, 1980). A potential marker for choline deficiency is the phosphatidylcholine level found in plasma.

Phosphatidylcholine is present in many cells as part of the phospholipid component in the membranes, especially red blood cells. It is possible that this pool of membrane phosphatidylcholine is metabolized to maintain choline for acetylcholine synthesis in a choline deficient state. For these reasons, measurements of both plasma choline and plasma phosphatidylcholine may be useful in detecting changes in tissue free choline as a result of long duration physical performance.

Dietary Sources of Choline

Choline is found in a wide range of plant and animal foods. For example, eggs, liver, red meats, fish, peanuts, peanut butter, spinach, cauliflower, iceberg lettuce, and whole wheat bread are all good dietary sources of choline. An average daily intake of choline is about 400 to 900 mg (Ziesel, 1981). It is believed that normal American diets deliver adequate choline.

Choline and Human Performance

Although choline is not considered an essential nutrient by humans at this time, ongoing investigations on the physiological, biochemical, and pharmacological effects of choline to meet known or suspected human needs are still being explored. An important area of choline research deals with the administration of choline and its subsequent accelerated synthesis and release of the acetylcholine by neurons (Zeisel and Blusztajn, 1994).

Acetylcholine is an important neurotransmitter controlling such diverse neural functions as memory and control of muscle function. The choline/acetylcholine relationship appears to be important during exercise because:

- a) with prolonged neuromuscular efforts, the depletion of plasma choline might lead to fatigue due to insufficient availability of acetylcholine, while increasing plasma choline exogenously might enhance acetylcholine availability for neuromuscular transmission. Experimental studies in animals suggest that depletion of acetylcholine may contribute to fatigue produced by sustained electrical stimulation (Krnjevic and Miledi, 1959; Liley and North, 1952; Pagala,

Namba, and Grob, 1984);

- b) plasma choline concentrations in elite runners were lowered after competition (Conlay, Wurtman, Blusztajn et al., 1986; Wurtman and Lewis, 1991; Sandage, Sabounjian, White, and Wurtman, 1992), in eight marines after running on a treadmill at 12 km/h for over 2 hours (Horn, Allwörden, and Feldheim, 1993), and in humans consuming a choline-free diet (Goldberg and McCaman, 1973);
- c) reductions of serum choline have been found to be associated with a slowing in transmission of the contraction-generating impulse across muscle (Xia, 1991).

There are two reports suggesting that long distance running will cause a fall in plasma choline levels. In a study of 17 experienced runners competing in the Boston Marathon, Conlay, Wurtman, Blusztajn et al., 1986 found approximately a 40% decrease in plasma choline levels compared to levels usually observed in fasting subjects. In another report, Sandage, Sabounjian, White, and Wurtman (1992), nine male runners showed a similar reduction in plasma choline of approximately 30% (from 11.3 ± 1.3 to $8.2 \pm 0.7 \mu\text{mol/L}$) after a 20 mile run. This crossover study also supplemented subjects with a beverage containing either choline citrate or a placebo. Both beverages were standardized and taken in a double but equal dose 1 hr before, and half-way through the run. Supplementation with the choline beverage prevented a second fall in plasma choline and improved the subjects' run times an average of 5 mins. There have been no reports of differences observed following runs of shorter distances (Sabounjian, unpublished).

A study performed by Burns, Costill, Fink et al. (1988) looked at the influence of two levels of choline from soy lecithin on plasma choline levels and exercise performance of ten trained cyclists. The ten cyclists exercised on a cycle ergometer for 105 mins at an intensity equal to 70% $\dot{\text{V}}\text{O}_2$ max, followed by an all out, self-paced 15 min performance ride. The cyclist followed three randomly ordered trials after feedings of 0.0 g, 1.1 g, and 1.8 g of choline per day. There was a dose response increase in serum choline levels to the different choline feedings, but no corresponding improvement in performance.

More recently, Spector, Jackman, Sabounjian et al. (1995) investigated the relationship between plasma choline levels and the development of peripheral fatigue in subjects performing brief, supra maximal and prolonged cycling tests. In the prolonged group, ten subjects performed continuous cycling equivalent to 70% of their $\dot{V}O_2$ max at a cadence of between 80 to 90 rpm until exhaustion. They received a 200 cc fruit drink supplemented with 2.43 g choline bitartrate (1 g free choline) 45 mins prior to the start of their cycling and a second dose equal to their first dose 25 mins after onset of exercise. In the prolonged group, while receiving the placebo beverage, the change in plasma choline level from pre- to post-exercise was negatively correlated to riding time, indicating that subjects riding for longer periods tended to have greater decreases in plasma choline concentration. Choline ingestion did not significantly affect exercise performance, although there were consistent, positive non-significant effects of choline on performance across conditions. The average time to exhaustion was 74.8 mins after choline ingestion and 71.9 mins with placebo ingestion. Neither riding time to fatigue nor total work output was significantly modified by oral administration of quantities of choline sufficient to produce elevations in blood choline levels.

A review of the literature examining the effects of choline supplementation on strength uncovered a single report. A study utilizing a blind, crossover design with unsupplemented controls was performed by Stanton (1951). Grip strength was tested before and after 2 weeks of daily supplementation with 30 g of lecithin (approximately 4.5 to 6 g of free choline, assuming a 20% phosphatidylcholine concentration). No increases in strength were noted.

Almost all circulating choline (98% to 99%) is found in phosphatidylcholine associated with lipoproteins. Phosphatidylcholine is a required component of the very low-density lipoprotein (VLDL) particle (Yoa & Vance, 1988 and 1989). Very low-density lipoprotein is very active in transporting triglycerides from endogenous storage sites to exercising tissue in such endurance events as marathon running. Also, the observed fall in plasma choline may have been related to an increase in plasma high-density lipoproteins, thus creating a plasma choline drop (Thompson, Cullinane, Henderson et al., 1980). Neither plasma VLDL or high density lipoproteins (HDL) were measured in previous studies.

Rationale for Choline

It has been proposed that decreases in plasma choline levels may impair acetylcholine release at the neuromuscular junction, resulting in impaired human performance. However, there are no studies to date that have shown a decreased serum choline level to significantly affect human performance. In subjects fed a choline-free diet, a 40% reduction in plasma choline was associated with a slowed transmission of the concentration-generating impulse across the skeletal muscle, although no impairment in neuromuscular transmission was observed (Xia, 1991). Based on experimental studies of depletion of acetylcholine with repetitive neuromuscular stimulation (Krnjevic and Miledi, 1959; Liley and North, 1952), it is conceivable that efforts which activate slow or fast twitch motor units repetitively may lead to depletion of acetylcholine and, consequently, to neuromuscular fatigue, as well.

The neurotransmitter acetylcholine is synthesized from precursors normally obtained from dietary sources of choline. The levels of this nutrient in the brain control the rates at which their neurotransmitter product is synthesized. Dietary control of neurotransmitter product synthesis is possible because:

- 1) the biochemical precursors for this class of neurotransmitters readily gain entry into the brain (Wurtman, Hefti, and Melamed, 1981);
- 2) the rate limiting enzymes in the neurotransmitter synthesis pathway are not saturated with precursor-substrate at physiological concentrations (White and Wu, 1973; Blusztajn and Wurtman, 1983);
- 3) these neurotransmitters do not "feedback" to inhibit their own synthesis (Wurtman and Wurtman, 1985).

Many studies (Haubrich, Wedeking, and Wang, 1974; Wurtman, Hirsh, and Growdon, 1978; Hirsch, Growdon, and Wurtman, 1978; Zeisel, DaCosta, Franklin et al., 1991) have shown that both lecithin and choline supplements can increase blood choline levels. Previous reports also pointed out that elevations in plasma choline have not been shown to decrease acetylcholine, nor is there reason to believe that

elevated plasma choline levels would impede performance.

Choline and Mental Performance

Modulating cholinergic neurotransmission affects learning performance. Anticholinergic drugs such as scopolamine interfere with acquisition of both active (Meyers, Roberts, Riciputi, and Domino, 1964) and passive (Meyers, 1965) avoidance learning tasks in rats. Scopolamine also impairs performance on a serial learning task in humans (Sitaram, Weingartner, and Gillin, 1978). In contrast, choline supplementation may improve learning performance in a variety of species, including human (Sitaram, Weingartner, Caine, and Gillin, 1978; Sitaram, Weingartner, and Gillin, 1978). The administration of choline increases brain levels of acetylcholine (particularly following pre-treatments) that increase firing rates of cholinergic neurons (Wecker, 1986, 1988; Miller, Greenblatt, Roy et al., 1989), which may result in improvements in learning performance. This study will assess cognitive performance and subjective states before, during, and after an exercise task designed to reduce plasma choline levels. These dependent measures will be compared following exercise with choline supplementation to baseline values following exercise without choline supplementation.

Choline and Lecithin Administration

The Select Committee on Generally Regarded as Safe (GRAS) Substances (1979) evaluated the health aspects of choline and lecithin as added food ingredients and concluded that there is no hazard to the public when either is added to the food in the usual amounts. The GRAS designation indicates general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. According to an ad hoc review by participants in the Life Sciences Research Office (LSRO), Washington, DC, self-administration of supplemental dosages of choline or lecithin appears to be self-limiting, and lasting health hazards from prolonged self-administration appear improbable (Wood and Allison, 1982). The Select Committee commented that available evidence raises no suspicion that choline salts have harmful effects at dosage levels that are several orders of magnitude greater than the most generous current estimates of human intake.

The LSRO participants agreed that 16-20 g/day of choline chloride (11.9 to 14.9 g free choline) approximated the highest tolerable dose. The ad hoc review reported that 60-80 g of lecithin (85% phosphatidylcholine) have been given to some patients without difficulty or side effects. In one study, six human subjects ate three meals a day containing lecithin equivalent to 5 g/day of free choline for 2 consecutive days. The diets were well tolerated and no adverse reactions were reported (Hirsch, Growden, and Wurtman, 1978).

The plasma response to dietary choline and lecithin are very different. Supplements containing up to 3 g of choline chloride have been used by Hirsch, Growden, and Wurtman (1978) in adults to examine the relation between dietary choline and serum choline levels. Three grams of choline chloride caused an 86% rise in serum choline, attaining peak values after 30 mins and returning to baseline within 4 hrs. The equivalent amount of choline in the form of lecithin caused a 33% rise in serum choline after 30 mins, but levels continued to rise until peak values of 265% over control were reached after 12 hrs. Choline supplementation had no effect on serum glucose, insulin, cortisol, or prolactin levels. Lecithin, unlike choline salts, will cause an increase in serum triglyceride levels (Hirsch, Growden, and Wurtman, 1978). Choline supplementation approximating 20 g of pure choline base has caused diarrhea and foul-smelling intestinal gas production (Chan, 1991). The peripheral cholinergic effects of large doses of choline (greater than 20 g/day) are nausea, vomiting, salivation, sweating, and anorexia (Wood and Allison, 1982).

The distance runners examined in the study by Sandage, Sabounjian, White, and Wurtman (1992) and the cyclist examined by Spector, Jackman, Sabounjian et al. (1995) were supplemented with choline citrate at a dose of 2.8 g (1 g of free choline) 1 hr before their performance and an identical dose after completing half of their 20 mile run. These authors have used similar doses in unpublished studies with no side effects. In the study by Burns et al. (1988), cyclists were supplemented with soy lecithin equal to as much as 1.8 g of choline without any reported side effects.

CHAPTER TWO

OBJECTIVES

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INTRODUCTION

The data from two previous uncontrolled studies (Conlay, Wurtman, Blusztajn et al., 1986; Sandage, Sabounjian, White et al., 1992) indicated that plasma choline may be decreased after prolonged exhaustive aerobic exercise, particularly running. Even under the controlled conditions found in the study by Spector, Jackman, Sabounjian et al. (1995), they concluded that there is some indication that the duration of an exercise is important in the depletion of plasma choline based on their observation that subjects ingesting placebo who rode the longest tended to have the greatest decrease in choline levels from pre- to post-exercise. There is a need to determine if plasma choline levels are also decreased after participating in a physically demanding military relevant task under controlled conditions. Another important element related to soldier mobility is the issue of fatigue caused by the energy expended in prolonged load carrying and marching. Soldiers must be capable of performing high levels of anaerobic work using muscular strength and endurance after prolonged aerobic activity.

A possible explanation for the variation observed in plasma choline response to performance is that the neurophysiological response by runners, cyclists, and heavy backpackers are all different. The degree of plasma choline depletion may be specific to the activity being performed. Thus, a prolonged bout of heavy load carriage which simultaneously involves both types of motor units, both aerobic and anaerobic work, may have an entirely different effect on changes of plasma choline and the performance response after choline supplementation.

The primary objectives of this study were to determine the effects of choline supplementation on work production, level of fatigue, mood, and plasma choline levels of soldiers after heavy load carriage work. A choline beverage supplement and a placebo-control beverage were administered to a group of soldiers engaged in heavy load carriage work. This task was chosen because it is one of the most common and most physically demanding tasks routinely performed by SOFs and combat units. Because our exercise task was of a considerably longer duration, we increased the choline supplementation to 8.4 g of choline citrate (3 g of free choline) per treatment.

The objectives of the study were:

1. To determine whether performing a military endurance activity such as a 20 km load carriage march on a motorized treadmill with a 29.6 kg rucksack within 4 hrs would cause a decrease in plasma choline.
2. To evaluate the ability of a choline supplement to maintain strength and endurance measured immediately following a moderately heavy load carriage exercise test.
3. To examine the effects and interactions of a choline supplement and sustained load carriage on mood, cognitive performance, hand/eye coordination and fine motor control.
4. To determine whether supplemental choline would affect ratings of perceived exertion of soldiers performing a single bout of sustained heavy load carriage exercise.

We hypothesized that choline supplementation treatment would:

- (1) increase plasma choline levels,
- (2) increase time to exhaustion on a post-test treadmill run (aerobic endurance),
- (3) result in a decreased rating of perceived exertion and improved perception of vigor and energy,

- (4) increase muscular endurance,
- (5) preserve rifle marksmanship accuracy following exhaustive aerobic exercise.

The changes noted were in comparison to those achieved from the placebo treatment. The proposed study used a choline supplement dose equivalent to 6 g free choline. There were two potential delivery options: (1) to provide a single dose of lecithin (phosphatidylcholine) incorporated into a commercially-made candy bar base approximately 3 hrs before the start of treadmill exercise; or (2) to give via a beverage in two equal doses: the first dose, 8.4 g of choline citrate (3 g free choline) 30 mins prior to the start of the treadmill exercise, and another 8.4 g choline citrate (3 g free choline) approximately 1 hr prior to the completion of the treadmill march around the 14 km (9 mi) point. Because oral lecithin as a source of choline takes longer to metabolize and to achieve peak levels with potential variation within subjects, it was preferable for the purposes of this short-term study to use an oral choline compound such as choline citrate or bitartrate that was metabolized more quickly.

MILITARY RELEVANCE

The ability of soldiers to carry heavy loads has been an important part of our past military history and will continue to be critical to our future success in the combat environment. Soldiers must be capable of performing high levels of anaerobic activity (fighting) once they make contact with the enemy. In the words of Marshall (1950), a soldier "is given great weights to carry, but unlike a pack animal or vehicle, his chief function in war does not begin until the time he delivers that burden to the appointed ground."

The physical demands placed on today's SOFs units are greater than those experienced by their predecessors. They must be prepared for lengthier insertions and relatively long periods at or near a target, requiring heavier loads to be carried to support longer mission durations. Training requirements have been extended to prepare for these greater varieties of missions and contingencies. Optimal nutritional status is strongly associated with maximal physical and mental performance.

Ongoing scientific investigation into nutritional strategies have targeted potential performance enhancing nutrients. Choline supplementation is reported to improve aerobic endurance and reduce post-run fatigue. Through the Performance Enhancing Ration Components (PERC) joint working group, we are researching specific nutrients to investigate whether or not supplementing garrison or field rations with dietary additives such as choline enhance the physical performance of our combat fighting forces. This study assessed possible beneficial effects of choline on physical and mental performance.

CHAPTER THREE

THE EFFECT OF CHOLINE SUPPLEMENTATION ON PHYSICAL PERFORMANCE

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OVERVIEW

Up to 20 male military volunteers (18 to 35 years old) were recruited for the study and participated in a 20 km road march with up to a 65 lb rucksack on two different occasions to determine the effect of choline supplementation on mental and physical performance.

STUDY VOLUNTEERS

This study was conducted with 14 male service members (18 to 35 years old) who were recruited for the study from the 75th Infantry (Ranger) Regiment, Fort Benning, GA. This study was restricted to males, as choline supplementation is initially being targeted for SOFs and combat fighting units which are almost entirely male. If

beneficial effects are observed, future studies would include female subjects. Each potential subject was screened, using a medical history and physical examination, for any condition which would preclude safe participation in the study or interfere with data interpretation (prior heat injury, history of foot and joint injuries, back problems or other musculo-skeletal problems, cardiovascular abnormalities, high blood pressure). The medical examination and review of records was done at the recruitment site by a physician. There was a minimum selection body weight of 64 kg. This study was conducted in a fixed facility that was able to control the environmental conditions for all volunteers. The volunteers received verbal and written explanation as to the nature, duration, purpose, risks, and benefits of the study and elected to volunteer by signing a Volunteer Agreement Affidavit (Appendix A).

EXPERIMENTAL DESIGN

This study employed a crossover, double-blind design, and was conducted over 2 days with a washout period of 1 week between individual tests. The data collection sequence is presented in Table 3.1. Data collection was identical for both study days, and the specific time-line for the data collection followed on test days is shown in Table 3.2. Two days prior to testing and the day of testing, each subject maintained an estimated food record. Food consumption was allowed up to 3 hrs prior to testing and then nothing by mouth except for the consumption of ad libitum water. The subjects were given either a placebo (non-nutritive) beverage or an identical treatment beverage (non-nutritive) supplemented with choline 30 mins prior to the start of the load carriage test, and again, near the 14 km (9 mi) point of the march. The choline treatment beverage was given in two equal doses (8.4 g choline citrate equivalent to 3 g free choline per treatment), totaling 6 g of free choline.

Table 3.1 Sequence of data collection.

	Screening	Pre-Load Carriage Test	Load Carriage Test	Post-Load Carriage Test
Briefing/Consent	X			
Medical Clearance	X			
Anthropometric	X			
Questionnaires	X			X
VO₂ max	X			
Food Record		X		
Treatment (placebo or choline)		X	X	
Run-to-Exhaustion				X
Mental Performance	X (training)	X	X	X
Borg Perceived Exertion			X	X
Oxygen Consumption			X	
Blood Draw		X		X
Noptel (Marksmanship)	X (training)	X		X
Strength/Endurance Test	X (training)			X

Table 3.2 Choline study time line.

TASK	TIME
2-Day Food Record	Turned in upon arrival to lab and reviewed
Blood Draw	L - 40 mins prior to start of load carriage test
Treatment	L - 30 mins prior to start of load carriage
POMS/Mental Performance	L - 25 mins prior to start of load carriage
<u>Begin Load Carriage</u>	<u>L + 0 minutes</u>
Mental Performance	L + 35 mins and +1 hr 35 mins
Oxygen Consumption/Heart Rate	L + 40 mins and + 1 hr 40 mins
Perceived Exertion	L + 50 mins and + 1 hr 50 mins
Mental Performance	L + 2 hr 35 mins and +3 hr 35 mins
Oxygen Consumption/Heart Rate	L + 2 hr 40 mins and + 3 hr 40 mins
Perceived Exertion	L + 2 hr 50 mins and + 3 hr 50 mins
Treatment	L + 2 hr 50 mins or 9 mile marker
End Load Carriage	L + 3 hr 50 mins
Run-to-Exhaustion	L + 4 hrs
Blood Draw	L + 4 hrs 10 mins, directly following the Run-to-Exhaustion
Marksmanship	L + 4 hrs 20 mins
Strength/Endurance	L + 4 hrs 30 mins
POMS/ Mental Performance	L + 4 hrs 45 mins

L - Time prior to (-) or after start of (+) load carriage testing

METHODS

Medical Clearance

Volunteers for the study were medically cleared by MAJ (Dr.) Amoroso a few days prior to the study. As previously mentioned, those individuals with medical conditions that would preclude participation in the study because of temporary medical illness or orthopedic injury were excluded.

Anthropometric Data and Body Composition

Pretest vertical height was measured in duplicate to the nearest 0.1 cm using a stadiometer (Holtain Stadiometer, Holtain, Ltd.). Standing height was measured in stocking feet and standing on a flat surface, feet together, and knees straight. The subject's head, shoulder blades, buttocks, and heels were in contact with a vertical wall. Body weight was measured using a calibrated electronic battery-powered scale (e.g., Model 6800, Cardinal Detecto) accurate to 0.1 kg. Seven skinfold thicknesses were measured by a single observer (chest, midaxillary, triceps, subscapular, abdomen, suprailiac, and thigh) and percent body fat was calculated as described by Jackson and Pollock (1985).

Maximal Oxygen Uptake

Maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) was determined using a discontinuous, progressive treadmill protocol (Mitchell, Sproule, & Chapman, 1957). The subject began with a warm-up at 6 mph, 0% grade for 6 mins. After a brief rest period, the subject performed two to four bouts of exercise with the speed held constant between 6 and 8 mph. The grade was increased every 3-4 mins in increments of 2.5% until a plateau was seen in oxygen uptake (defined as less than a $0.15 \text{ l}\cdot\text{min}^{-1}$ increase with a 2.5% increase in grade). Oxygen uptake was measured using a computerized measurement system consisting of a Hewlett-Packard 9000 Model 300 computer, a turbine flowmeter (KL Engineering Co., Sylmar, CA) for expired volume and an Applied Electrochemistry S-3A Oxygen analyzer (Ametek, Pittsburgh, PA) and Beckman LB-2 CO₂ analyzer (Sensormedics Inc, Yorba Linda, CA). Heart rate was determined

electrocardiographically throughout the test.

Food Intake

Food intake data were collected by the volunteers with estimated food records for 48 hrs prior to each test. Subjects were instructed by a registered dietitian and provided with specific verbal and written instructions and procedures for reporting detailed dietary intake. Food records were examined by the registered dietitian to ensure that all relevant information was included on the dietary forms. Food records were analyzed using Nutritionist IV, Version 4.0 nutrient analysis software (N-squared Computing, First Databank, The Hearst Corporation, San Bruno, CA). This software contains a currently updated database of over 13,000 foods including many brand name manufacturer's items and products from national fast-food restaurants. The nutrient data are based primarily on all available USDA data and scientific journal and industry sources. Subjects were instructed to consume their normal diet. Total energy intake, macronutrient consumption and dietary levels of various vitamin and minerals were estimated. Subjects were asked to avoid consumption of the following high dietary sources of choline 72 hrs prior to the load carriage test: whole eggs, liver, peanut butter, peanuts, whole wheat bread, cauliflower, spinach and iceberg lettuce. One subject failed to return a food record form before his second testing, thus his data was not included in the analysis.

Demographics, Daily Record, and Final Questionnaire

Demographics and background information were determined using a questionnaire (Appendix B). This questionnaire was self-explanatory, and it was administered after the volunteers were briefed and they had signed the informed consent form. Filling out this questionnaire took no longer than 5 mins. A data collector was available for assistance and for reviewing the form upon completion. The data collected were used to describe the sample volunteers participating in the study. A daily record questionnaire (Appendix C) was administered at the completion of the last post treadmill performance task. Each soldier completed a daily record questionnaire which asked them 1) about their performance, 2) if they thought they received the placebo or the choline supplemented beverage, 3) how much they liked or

disliked the treatment beverage they received, and 4) if they had any physical symptoms that they attributed to the treatment beverage they received. Completing the questionnaire took about 2 mins. The Final Questionnaire (Appendix D) asked the soldier to rate the differences between the two treatments regarding taste and performance. Completing this questionnaire took less than 2 mins.

Treatment

One-half hour prior to the load carriage treadmill exercise, each volunteer received either the placebo or the choline citrate treatment. The specially formulated beverages (placebo and treatment) were designed by the Technology Acquisition Division of the Sustainability Directorate at Natick RD&EC. The placebo and choline citrate beverages were identical in appearance, smell, taste, and texture. The concentration of choline in the choline citrate beverage was equivalent to 6 g of free choline. The amount of beverage or energy content did not vary based upon subject's size. The formulation should not cause any side effects based on the relatively low concentration of choline citrate being administered.

The beverage was sweetened with aspartame. It was flavored with a commercial Lemonade Kool-Aid mixture. The proposed ingredient breakdown was similar to other non-nutritive beverage mixtures used in other studies, a combination of aspartame and Kool-Aid (Kool-Aid: unsweetened, fortified with vitamin C, artificially colored and flavored.) The ingredient breakdown of the treatment and placebo drinks is shown in the chart below.

Treatment			Placebo		
Ingredients:	%	g/pint	Ingredients:	%	g/pint
Aspartame	0.014	0.071	Aspartame	0.01	0.071
Lemonade Flavor Kool-Aid	2.60	12.9	Lemonade Flavor Kool-Aid	2.64	12.9
Choline Citrate	1.69	8.4	Citric Acid	0.24	1.2
Maltodextrin	0.20	1.0	Maltodextrin	0.20	1.0

The effects of a choline supplement on plasma choline levels have been studied by Hirsch, Gowden, and Wurtman (1978). Within 30 mins after consuming a choline

supplement, plasma levels had risen by 82% and began to return to control levels within 4 hrs. Because of the use of a choline citrate supplement and its relatively short plasma half-life, a second treatment beverage was given during the 10 min break from the treadmill march, approximately 2 hrs and 50 mins from the start of the treadmill march. This was done to help ensure a prolonged elevation of plasma choline throughout the entire testing period. This is similar to the procedure followed in the study by Sandage, Sabounjian, White, and Wurtman (1992) and Spector, Jackman, Sabounjian et al. (1995) who used a double treatment of choline supplement in subjects running and cycling for a prolonged period of time.

Physical Performance

During the physical performance testing, a medical monitor and medical emergency equipment was on a short-range call, and located in the building where testing was being conducted to assist with any medical emergency.

Load Carriage Test

The load carriage test was performed on a motorized treadmill. Subjects walked at a rate of 5.6 km/h (3.5 mph), 3% grade for 4 hrs (with a 10 min rest period after each 50 min) carrying a total load 34.1 kg (75 lb) under both treatment conditions: with choline and without choline (placebo). This added load is the standard weight for a SOF soldier who is tested annually in a similar field endurance event. The standard Army load carrying system designated as all-purpose, lightweight, individual carrying equipment was utilized. This consists of the LC-2 frame to which the rucksack attaches. The frame is compatible with the standard equipment belt to which such items as the canteen, ammo pouches, etc. attach. Approximately 29.6 kg (65 lbs) was carried in the rucksack with the remaining 4.5 kg (10 lbs) distributed on the body (includes physical training uniform, running shoes and items on the belt).

The order of testing was randomized with 6-7 days separating load carriage sessions. The rucksack was individually packed by the subject, with the weight distributed as evenly as possible about the center of gravity.

The energy cost (oxygen uptake) of the load carriage test was determined between mins 40 and 50 of each hour using the portable Oxylog system. The subject breathed through a mouth/face mask and T-shaped, two-way, non-rebreathing valve (Hans Rudolph, Inc., Kansas City, MO) into respiratory tubing connected directly to the Oxylog which was attached to the side of the treadmill. The Oxylog consists of a turbine flowmeter to measure inspired ventilation volume and two polarographic sensors to measure inspired and expired oxygen concentrations. The Oxylog has been shown to be an accurate and reliable system for the measurement of oxygen uptake with reported differences of less than 5% compared to the Douglas bag technique (Louhevaara and Ilmarinen, 1985; Harrison, Brown, and Belyavin, 1982). During this same time period, heart rate was determined by Polar Pacer Heart Rate Monitor (Polar USA, Stamford, CT).

Blood samples were taken at two points during the treadmill protocol: prior to beginning the exercise, and after the run-to-exhaustion. The blood samples were taken by percutaneous venipuncture of a forearm vein by a USARIEM credentialed phlebotomist. The blood draw was conducted as outlined in USARIEM standard operating procedures. Water was available during the protocol and provided to subjects upon their request.

Perceived Exertion

Ratings of perceived exertion were obtained from each soldier during and at the completion of the load carriage exercise using the Borg scale (Borg, 1973). The Borg scale consists of a 15-point continuous scale from 6 to 20 with each odd number anchored by a verbal expression of difficulty ranging from "very, very light" to "very, very hard." Each soldier's perceived exertion was assessed at every mile completed after the consumption of placebo or choline treatment beverage beginning at the mid-exercise break. At the completion of the load carriage test, the soldiers were asked to rate their overall perceived exertion.

Aerobic Endurance

Upon completion of the load carriage exercise, each subject was evaluated for endurance performance using a treadmill protocol. Subjects removed the entire load carriage system. An adapted treadmill protocol was followed starting with an initial intensity of 8 km/h, 3% grade for 2 mins. Thereafter, intensity was increased by increasing speed only, 1.6 km/h every 2 mins until the subject could no longer continue (volitional limit to continuing). The total time on the treadmill and maximal heart rate achieved were recorded and used for comparison between placebo and choline supplement trials.

Rating of Treatment and Placebo

At the completion of the treadmill exercise and all other post-test physical assessments, each soldier completed a questionnaire asking them (1) about their performance, (2) if they thought they received the placebo or choline supplemented beverage, (3) how much they liked or disliked the supplemented beverage they received (The questionnaire asked the soldiers to rate the beverage supplement they received on the test day using a nine point hedonic scale ranging from 9 = "like extremely" to 1 = "dislike extremely.") The beverage supplement was rated in terms of overall acceptability, appearance, flavor, and after taste.), and (4) if they had physical symptoms that they attributed to the beverage supplement they received, including questions to ascertain the incidence of gastrointestinal symptoms such as stomach cramps or aches, nausea, gas, diarrhea, etc. Completing this questionnaire took less than 5 mins.

Blood Collection and Analysis

Prior to beginning the treadmill load carriage test and immediately after finishing or discontinuing the treadmill exercise bout, blood samples were collected by percutaneous venipuncture of a forearm vein. Blood samples were collected in two separate vacuum container tubes, red top SST and purple top EDTA (Tables 3.3 & 3.4). For the entire study, there were two treadmill load bearing tests (40 ml blood) over a period of 8 days. There was 7 days between each set of treadmill exercise

tests.

Table 3.3 Tube 1, 13 ml Red Top (SST).

Volume	Priority & Type	Label	Analysis	Laboratory
2 ml	1 S	CHEM-1	Alb, BUN, Ca, Cl, Chol, Crea, Fe, Fe-binding, Glu, HDL-C, K, Lactate, Mg, Na, P, SGPT, Trig, 3-hydroxybutyrate, CPK	USARIEM
2 ml	2 S	CHEM-2	[same as CHEM-1]	USARIEM

Table 3.4 Tube 2, 7 ml Purple Top, EDTA.

Volume	Priority	Label	Analysis	Laboratory
1 ml	2 P	EDTA-1	Plasma Choline	Equipped with Gas Chromatography/Mass Spectrometry
1 ml	3 P	EDTA-2	Phosphatidylcholine	Equipped with Gas Chromatography/Mass Spectrometry
1 ml	4 P	EDTA-3	Glycerophosphocholine	Equipped with Gas Chromatography/Mass Spectrometry

After the CBC was performed, the tube was centrifuged. Blood samples for choline, phosphatidylcholine, and glycerophosphocholine were placed on ice immediately after being drawn. Plasma was removed, processed, immediately frozen, and then stored at -80°C or colder prior to shipping for analysis of choline and its derivatives. Analysis of choline and its derivatives require a gas chromatography-mass spectrometry analyzer. Additional plasma was used to assay for cortisol, testosterone, triiodothyronine, thyroxine and thyroid-stimulating hormone. These endocrine markers

were analyzed at USARIEM using radioligand assay procedures.

Strength/Endurance Test

This test determined the decrement in strength/endurance as a result of the load carriage test and whether the treatment beverage affected any degradation. The barbell squat exercise was selected, as it involves muscles of the thigh, hip, and back, which are important for load carriage. The test requires the subject to squat with a 45.5 kg (100 lb) barbell at a metronome-cued rate that results in a power output of 100 W exerted by the lifter on the bar. The test conditions were empirically chosen as those for which the test should take no longer than 3 mins, even for a large, fit, test subject. Yet the weight is light enough for even smaller, less fit subjects to do at least a few repetitions. The following shows the derivation of the formula for calculating the metronome rate for each lifter based on the individual's measured vertical barbell travel per repetition.

$$\text{power}_W = \text{force}_N \times \text{distance}_{\text{m}/\text{repetition}} \times \text{rate}_{\text{repetitions/s}}$$

substituting

$$\text{force}_N = \text{mass}_{\text{kg}} \times 9.80_{\text{m/s}^2}$$

$$\text{rate}_{\text{repetition/min}} = \text{rate}_{\text{repetitions/s}} \times 60$$

and rearranging yields

$$\begin{aligned}\text{rate}_{\text{repetitions/min}} &= (100_W \times 60) / (45.5_{\text{kg}} \times 9.80_{\text{m/s}^2} \times \text{distance}_{\text{m}/\text{repetition}}) \\ &= 13.5 / \text{distance}_{\text{m}/\text{repetition}}\end{aligned}$$

For example, if the bar moves vertically 54 cm in a measured repetition, we enter the equation with 0.54 m and get a repetition rate of 25 per min at which the subject is to be tested. It is evident that a taller subject, who moves the weight further with each repetition, requires a slower metronome rate than a shorter subject. The test is discontinued when the subject fails to maintain the metronome-cued rate. The score

is the number of repetitions or the total work performed (weight x distance/repetition x repetitions). The test was administered to all test subjects prior to and at the completion of the maximal performance treadmill test. It is used routinely in Dr. Harman's human performance laboratory at the U.S. Army Research Institute of Environmental Medicine, Natick, MA.

STATISTICAL ANALYSES

Repeated measures analyses of variance (ANOVA) were used to assess differences 1) before exercise and before consuming the test beverage, 2) after exercise and after consuming the placebo drink, and 3) after exercise and after consuming the choline drink. Prior to the repeated measures ANOVA analyses, two different T-Tests were run on each variable. The first assessed order effect differences (DAY 1 vs DAY 2) of the post-exercise measures. Initially, the order the volunteers received each beverage was balanced; however, due to dropouts, eight received the placebo beverage first and six received the choline beverage first. No significant order effects were observed for any performance measure ($p>0.05$). The second T-Test was used to examine if there were any differences in the pre-exercise (rested), pre-beverage (before choline or placebo was consumed) administration for any of the performance measures. No significant differences existed ($p>0.05$). Since no differences existed in the pre-exercise measures over the 2 days, they were averaged for the repeated measures ANOVA. Post-hoc analyses to determine the location of significant differences were done using Tukey's Multiple Comparison Tests. Statistical significance was set at $p\leq 0.05$. Pearson Product-Moment Correlations were run to assess the relationship between changes in plasma choline (baseline minus post-endurance levels) and run time-to-exhaustion (seconds).

RESULTS

Table 3.5 presents the mean (\pm SE) values and ranges for the physical characteristics of the subjects.

Table 3.5 Physical characteristics.

VARIABLE	MEAN \pm SE	RANGE
Age, yrs	26 \pm 4	19 - 33
Height, cm	178.8 \pm 1.5	167.0 - 187.6
Body Mass, kg	82.7 \pm 3.3	63.3 - 106.8
Body Fat, %	11.1 \pm 1.0	6.5 - 19.5

The subjects had a considerably lower percent body fat than compared to a large, similarly aged Army population (Fitzgerald, Vogel, Daniels et al., 1986). Dietary records collected 2 days prior to each test are summarized in Table 3.6 and indicate no significant differences in carbohydrates, protein, fat, cholesterol and total caloric intake.

Table 3.6 Dietary analyses results for study groups.

Parameter	Pre-Choline	Pre-Placebo
Carbohydrates (g)	475.9 \pm 169.1	467.1 \pm 193.1
Fat (g)	99.6 \pm 34.4	91.8 \pm 39.9
Protein (g)	115.4 \pm 32.2	101.0 \pm 39.7
Cholesterol (mg)	333.3 \pm 203.2	228.2 \pm 129.3
Total Intake (Kcal/day)	3339.5 \pm 873.9	3071.8 \pm 1057.3
% Carbohydrate calories	58%	60%
% Fat calories	28%	27%
% Protein calories	14%	13%

No significant differences between conditions at p<0.05

The mean (\pm SE) values and ranges for physiological variables measured at maximal exercise are presented in Table 3.7.

Table 3.7 Maximal physiological data.

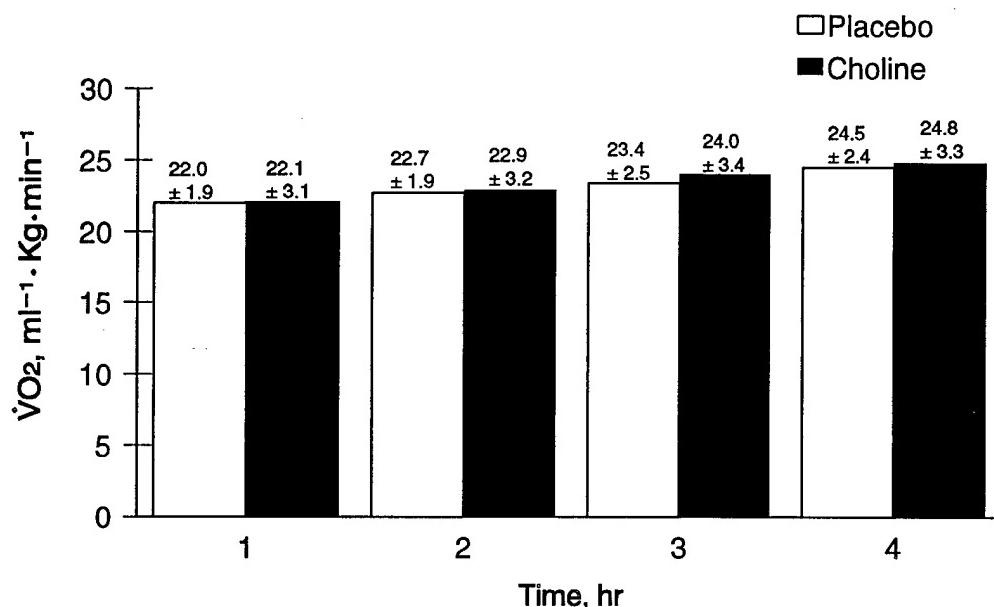
VARIABLE	MEAN \pm SE	RANGE
$\dot{V}O_2$ max, l·min ⁻¹	4.94 \pm 0.15	4.00 - 6.05
$\dot{V}O_2$ max, ml·kg ⁻¹ ·min ⁻¹	60.3 \pm 2.3	44.3 - 72.1
$\dot{V}E$, l·min ⁻¹ BTPS	160.7 \pm 5.5	123.1 - 194.2
Heart rate, b·min ⁻¹ ($\dot{V}O_2$ max Test)	190 \pm 1	182 - 200

The mean aerobic power for the subjects was considerably higher than that previously reported for a similarly aged Army population (Vogel, Patton, Mello, and Daniels, 1986).

The mean (\pm SE) oxygen uptake taken prior to the 10 min rest period for each hour of the load carriage test is shown in Figure 3.1. There was no difference between the choline and placebo treatments at any time during the 4-hr load carriage test. Oxygen uptake, however, did increase significantly ($p<.01$) over time for both treatments, agreeing with earlier studies of prolonged load carriage (Epstein, Rosenblum, Burstein, and Sawka, 1988; Patton, Kaszuba, Mello, and Reynolds, 1991).

A gradual increase in oxygen uptake during prolonged, submaximal, constant-rate exercise (known as $\dot{V}O_2$ drift), seemingly unrelated to either intensity or duration, occurs under a variety of exercise conditions (Saltin and Stenberg, 1964; Hagberg, Mullin, and Nagle, 1978; Kalis, Freund, Joyner et al., 1988). Factors postulated to account for this include increased body temperature, increased $\dot{V}E$, reduced mechanical efficiency, and a shift in substrate utilization (Casaburi, Storer, Ben-Dor, and Wasserman, 1987; Kalis, Freund, Joyner et al., 1988).

Figure 3.1 Load carriage test: oxygen uptake by type of drink consumed (mean \pm SD).



The mean data for heart rate and RPE for both treatments over the 4-hr load carriage test are shown in Table 3.8.

Table 3.8 Mean \pm SE for heart rate and RPE during the load carriage test.

Time, hr	HEART RATE		RPE	
	Placebo	Choline	Placebo	Choline
1	120±3	120±3	11±1	10±1
2	122±3	122±4	11±1	11±1
3	129±4	129±4	12±1	12±1
4	136±4	136±4	13±1	13±1

There was no difference between treatments for either heart rate or RPE at any time during the load carriage testing. However, both heart rate and RPE increased significantly over time ($p < .05$).

The energy cost (oxygen uptake) of the load carriage test was determined by the data collected between mins 40 and 50 of each hour using the portable Oxylog system. Using the relationship of work ($1 \text{ L O}_2 \approx 5 \text{ kcal}$), the mean energy expenditure showed no significant difference between trials. Following the choline treatment, energy expenditure was 1960 kcal as compared to 1935 kcal following the placebo treatment. The energy expenditure as a result of the treadmill load carriage task demonstrates a significant amount of work was performed prior to the performance tests being conducted.

Figure 3.2 presents the mean ($\pm \text{ SE}$) plasma choline levels prior to the load carriage test and following the endurance run-to-exhaustion for both treatments. The mean plasma choline level increased 128% ($p < .001$) above baseline levels in the choline supplemented group. There was no significant differences noted between levels from baseline and post-test results in the placebo group.

Figure 3.2 Plasma choline concentration prior to exercise & immediately after run-to-exhaustion *($p < .001$).

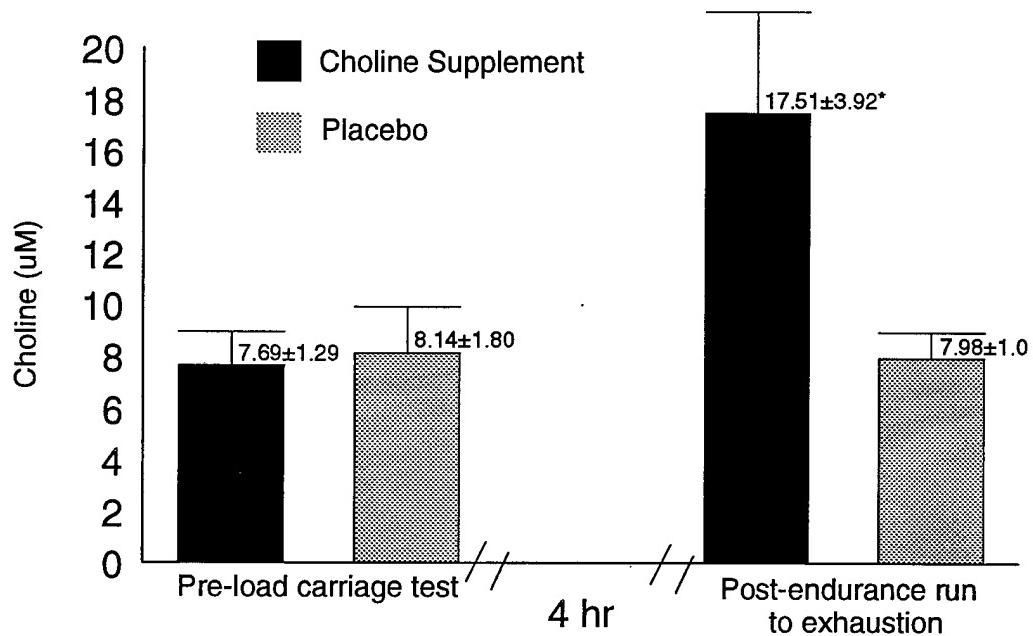
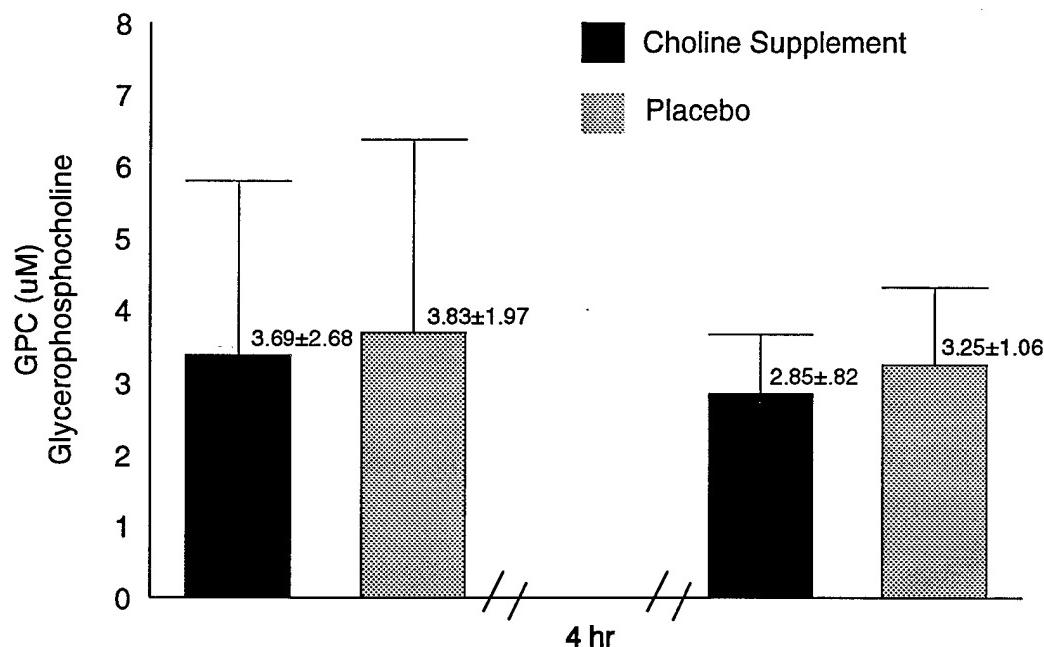
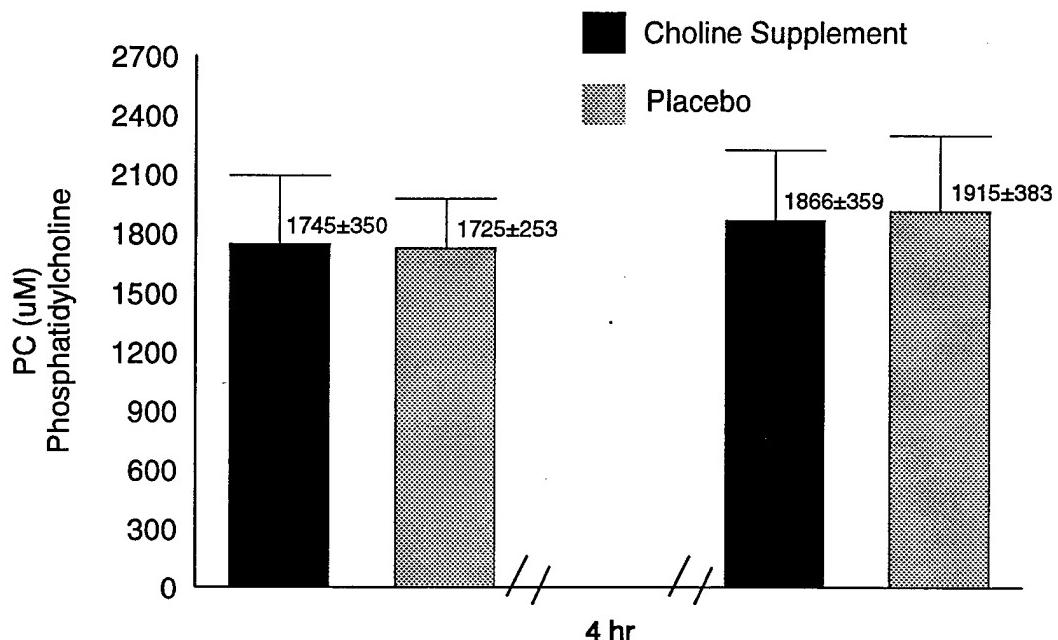


Figure 3.3 Plasma glycerophosphocholine concentration prior to exercise & immediately after run-to-exhaustion.



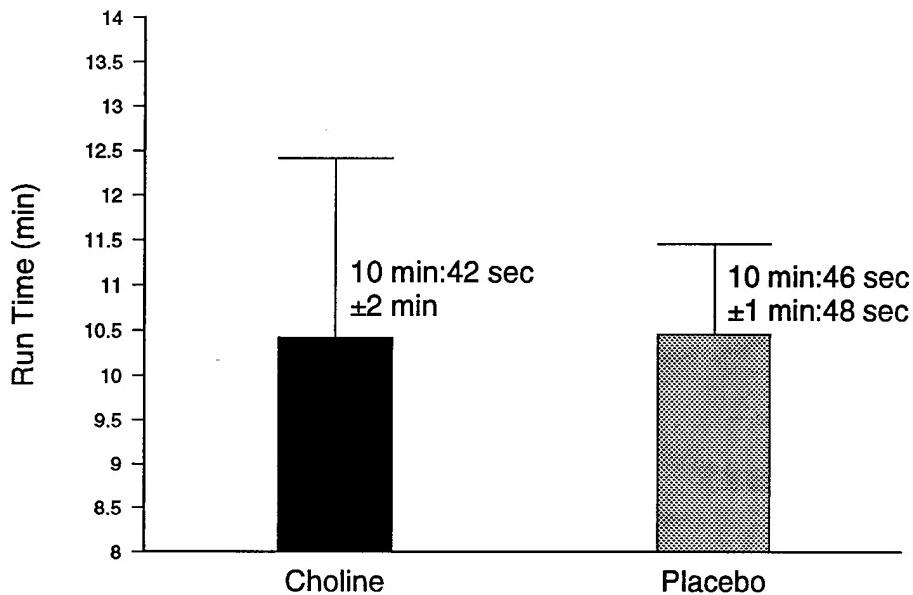
Figures 3.3 and 3.4 show no significant differences between levels of plasma glycerophosphocholine and phosphatidylcholine, respectively, from baseline to post-exercise levels.

Figure 3.4 Plasma phosphatidylcholine concentration prior to exercise & immediately after run-to-exhaustion.



Run-to-exhaustion time did not improve as a result of choline supplementation (Figure 3.5). It was hypothesized that choline supplementation would enhance endurance performance, especially after an exhausting task of prolonged load carriage.

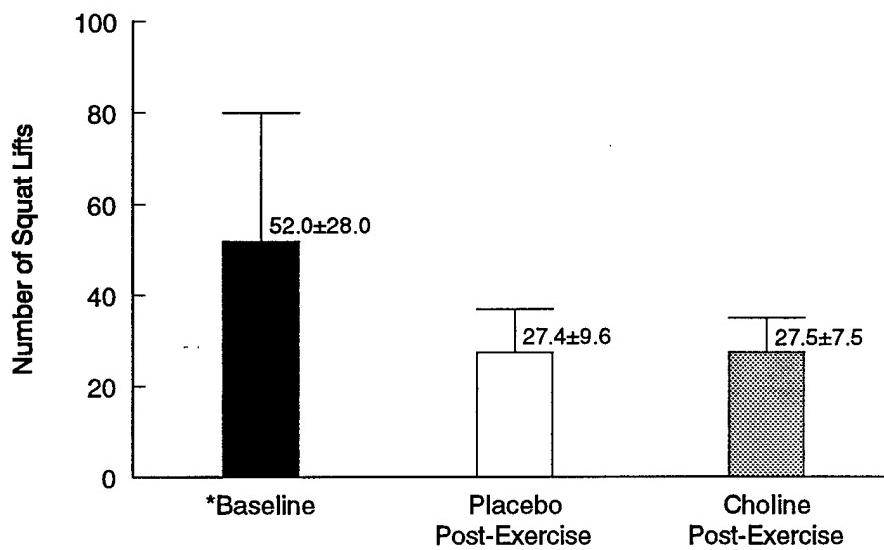
Figure 3.5 Post-load carriage test: Endurance run-to-exhaustion times.



Maximal squat performance declined by 53% of initial baseline measurement (Figure 3.6). The decline was almost identical for both treatments. Although this was a group of strong and highly trained individuals, the load carriage task caused a significant drop in lower extremity muscular strength that was not improved by choline supplementation.

Nonesterified fatty acids and β -hydroxybutyrate (Table 3.9) were shown to increase well above their normal reference ranges, which are in agreement with increased fatty acid mobilization. This mobilization was in response to the low aerobic intensity work being performed over a long duration. Another potential contributor is the result of the subjects fasting for 10-12 hrs prior to being tested. The subjects had a very small pool of readily available carbohydrate for energy use.

Figure 3.6 100 lb repetitive squat lift: Pre-load carriage test post-endurance run-to-exhaustion by type of drink consumed.



*POST-EXERCISE IS SIGNIFICANTLY LESS THAN BASELINE AT p<0.05
NO SIGNIFICANT DIFFERENCES POST-EXERCISE BETWEEN PLACEBO AND CHOLINE AT p<0.05

TABLE 3.9 Mean (\pm SD) values for exercise and cardiovascular risk blood chemistries.

Measure	Treatment	Reference	Pre-exercise	Post-exercise
Triglyceride (mg/100ml)	C	10-190	101.13 \pm 53.26	97.60 \pm 34.09
	P		93.67 \pm 40.74	104.53 \pm 34.81
Cholesterol (mg/100ml)	C	<200	172.87 \pm 62.84	192.57 \pm 59.26
	P		173.37 \pm 55.77	191.93 \pm 57.21
HDL-Cholesterol (mg/100ml)	C	>35	37.58 \pm 7.39	42.98 \pm 7.88
	P		38.19 \pm 8.01	42.53 \pm 8.96
Glucose (mg/100ml)	C	70-110	90.76 \pm 7.11	106.33 \pm 21.21
	P		85.37 \pm 10.00	102.63 \pm 22.71
Creatine Kinase (μ L/L)	C	55-170	186.23 \pm 100.12	280.90 \pm 134.57*
	P		163.90 \pm 103.89	283.80 \pm 151.66*
Creatinine (mg/100ml)	C	0.6-1.2	1.17 \pm 0.11	1.49 \pm 0.12*
	P		1.12 \pm 0.17	1.41 \pm 0.11*
Lactate (mg/100ml)	C	5-20	15.37 \pm 4.69	64.82 \pm 27.65*
	P		16.30 \pm 4.92	62.01 \pm 32.16*
Beta-Hydroxybutyrate (μ mol/L)	C	0.00-0.42	2.28 \pm 1.49	4.01 \pm 1.25*
	P		2.21 \pm 0.52	4.55 \pm 1.69*
Nonesterified Fatty Acids (mmol/L)	C	0.30-0.48	0.35 \pm 0.16	1.28 \pm 0.53*
	P		0.28 \pm 0.53	1.07 \pm 0.40*

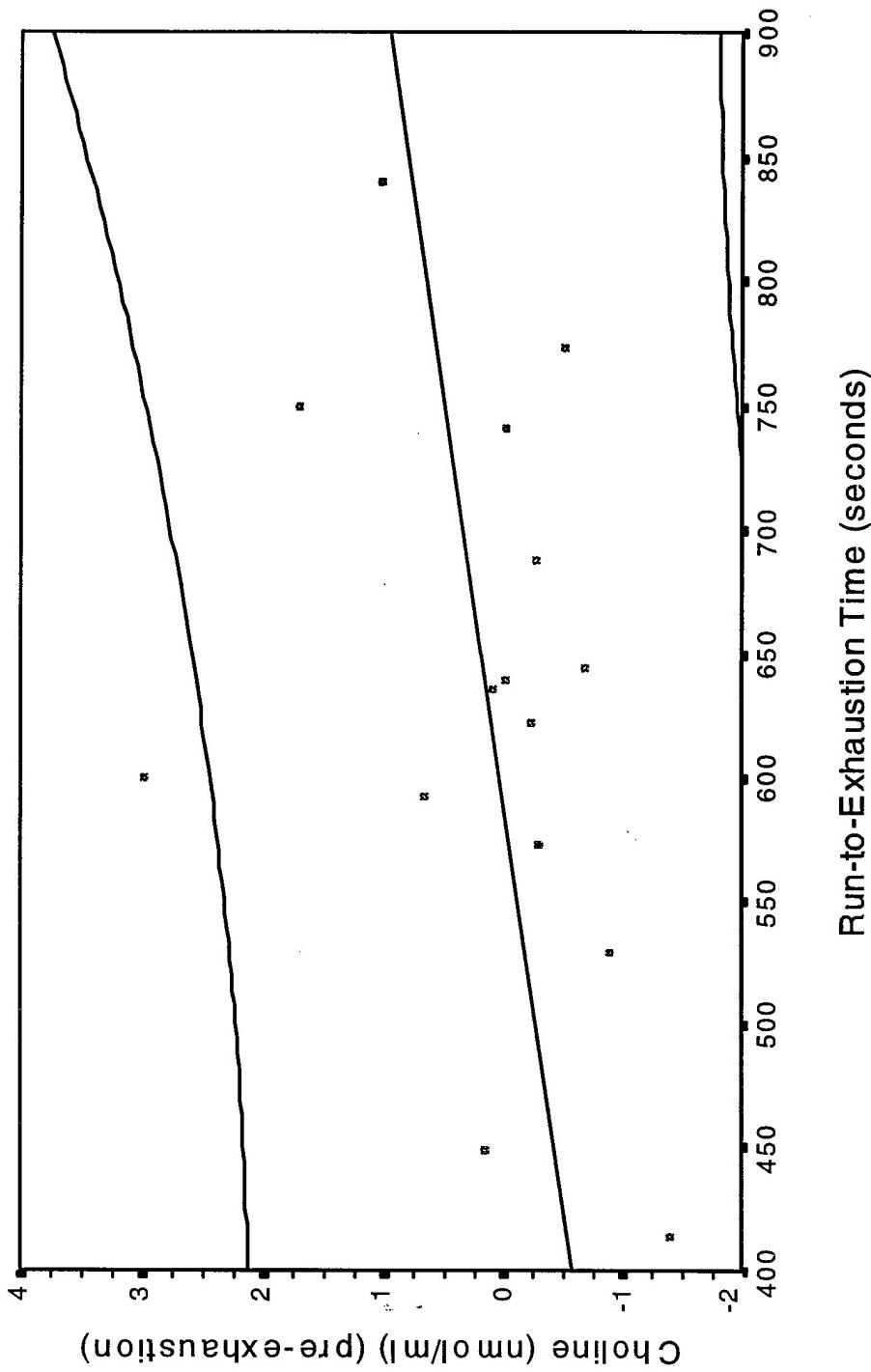
*Significantly different from baseline at $p \leq 0.05$, effect of time

Serum lactate concentrations were elevated above normal reference range. Lactate is the by-product of anaerobic glycolysis. It has been reported that to accept an aerobic effort as being near maximum, blood lactic acid levels should reach near 70 mg/100 ml or higher (McArdle, Katch, and Katch, 1986). The elevated levels of serum lactate after both treatments reflect the intense energy expenditure of the subjects during their run-to-exhaustion and suggest a near maximal effort. The lactate results probably do not reflect maximum levels achieved because there was a slight delay from the completion of the run-to-exhaustion to the final venipuncture as subjects were allowed to walk around briefly to cool down. Lactate is quickly cleared and taken up by the liver. Also, trained individuals such as the Rangers have a propensity to remove lactate at an accelerated rate over untrained individuals.

There was no significant decrease in plasma choline metabolites (phosphatidylcholine and glycerophosphocholine) concentrations. Esterified choline stores are used to maintain plasma choline levels as they become depleted. Because there was no drop in plasma choline levels, tissue levels represented by choline metabolites remained unchanged.

There is no indication that a more prolonged load carriage followed by a run-to-exhaustion endurance test would have resulted in a reduction of plasma choline. Subjects during the placebo treatment who ran the longest did not tend to have the greatest decrease in choline levels pre- to post-exercise (Fig. 3.7).

Figure 3.7 Correlation between the change (pre minus post) in plasma choline concentration and run-to-exhaustion time ($r=0.33$) for subjects receiving placebo beverage.



DISCUSSION

The present study investigated the relationship between plasma choline levels and the enhancement of physical performance following a prolonged exhaustive military relevant task. A major premise of this study was that increased plasma choline levels reflect the availability of the neurotransmitter acetylcholine at the neuromuscular junction, and that any fall in plasma choline levels reduces physical performance due to reduced synthesis of acetylcholine. No *in vivo* studies to date have investigated the relationship between modified plasma choline levels and concentrations of acetylcholine in skeletal muscle. Spector, Jackman, Sabounjian et al. (1995) commented that extrapolation of previous choline studies suggests that changes in plasma choline probably do reflect the amounts of acetylcholine in muscle available for neuromuscular transmission.

It is important to distinguish the effect of different types of exercise on choline depletion and choline influences on physical performance. Plasma choline levels in this study were evaluated during the performance of a highly specific military relevant task, consisting of a prolonged effort of heavy load carriage. Unlike other typical exercise regimens used to evaluate ergogenic supplements (e.g., running and cycling), it can be argued that the neurophysiological response of runners is different from cyclists with both being different from load carriage walking.

The Ranger volunteers in this study walked at a constant 3.5 mph at a 3% grade for 20 km. Their exercise heart rate during this effort started at 63% of their maximum and rose to approximately 72% of their maximum at the conclusion of the load carriage. This prolonged submaximal endurance effort was dependent on oxidative pathways for energy production, while the additional run-to-exhaustion continued to use the same pathway for most of each individual's efforts. During the subjects' run-to-exhaustion trials, subjects performed at 99% of their maximal heart rate level before fatigue, as shown by comparisons between run-to-exhaustion maximum heart rates and baseline $\dot{V}O_{2\text{max}}$ heart rates.

There were several important findings in this study. The first was that a 20

km load carriage march immediately followed by the run-to-exhaustion effort did not cause a reduction in plasma choline levels. This finding is not consistent with other reported studies. The study by Niels von Allworden, Horn, Kahl, and Feldheim (1993) in triathletes cycling at an average speed of $35 \text{ km}\cdot\text{h}^{-1}$ for 2 hrs demonstrated an average decline of plasma choline of 16.9%, which was reversed by administration of lecithin 60 mins prior to exercise. Spector, Jackman, Sabounjian et al. (1995) reported an average 9% decline in plasma choline in 10 male cyclists who rode over 80 mins. Conlay, Wurtman, Blusztajin et al. (1986) reported a 40% decline in mean plasma choline in 17 male subjects running the 1985 Boston Marathon.

In human performance studies, only two events have resulted in documented reductions in plasma choline: 1) running a marathon (Conlay, Wurtman, Blusztajin et al., 1986) and a 20 mile run (Sandage, Sabounjian, White, and Wurtman, 1992), and 2) cycling (Niels von Allworden, Horn, Kahl, and Feldheim, 1993; Spector, Jackman, Sabounjian et al., 1995). Yet only one study has shown any association between the depletion of plasma choline resulting in fatigue and a decrease in performance (Sandage, from a paper presented for Interneuron Pharmaceuticals Incorporated, 1992).

A common characteristic of these studies is the apparent influence of both intensity and duration on plasma choline. In this study, the duration of exercise was longer than all the previously cited studies except Conlay, Wurtman, Blusztajin et al. (1986) who studied marathon runners. But duration without intensity does not appear to be sufficiently intense to cause changes in plasma choline. If this is true, there are not many likely situations for an effective use of choline supplementation with a military application.

It is uncertain how long a soldier would have to perform a load carriage walk that might result in a detrimental reduction of plasma choline. Since the military travels on its stomach, it is very likely a soldier would be consuming components of an operational ration over the course of a prolonged load carriage movement. Operational rations may not provide many of the best sources of choline, but they would probably contain adequate amounts from the following two sources: 1)

phosphatidylcholine, found naturally as the choline containing phospholipid within meat and plant membranes found in most entrees and peanut butter spread, and 2) lecithin, used extensively as an emulsifying agent or antioxidant to aid in shelf-life stability of processed foods. There appear to be adequate choline resources to replenish any potential plasma choline losses.

In addition to the plasma choline assays, we also measured plasma phosphatidylcholine and glycerophosphocholine. Both are choline metabolites generally found in the membrane composition of cells. There were no significant changes in free plasma levels suggesting that cell membranes were not being metabolized to provide choline to maintain tissue free choline levels for potential acetylcholine synthesis.

Conlay, Wurtman, Blusztajn et al. (1986) theorized that because the majority of circulating choline is in the form of phosphatidylcholine associated with lipoproteins, a partial explanation for the observed drop in plasma choline in the group of marathon runners might be the result of an increase in plasma HDLs, creating a plasma choline "sink." They did not measure serum lipids in their subjects, but based this upon previous studies that looked at serum lipids before and after exercise, which suggested aerobic exercise produces higher concentrations of HDL (Alterkruse and Wilmore, 1973; Lopez, Vial, Balart, and Arroyave, 1974; Ratliff, Elliott, and Rubenstein, 1978; Leon, Conrad, Hunninghake, and Serfass, 1979; Thompson, Cullinane, Henderson, and Herbert, 1980). In fact the acute serum HDL changes reported by Thompson, Cullinane, Henderson, and Herbert(1980) in a group of male marathon runners was approximately 4 mg/dl greater after exercise compared to pre-race levels. Peak acute changes were recorded 5 min after exercise. We found similar changes in our study, a 5.4 mg/dl increase in the treatment group and 4.3 mg/dl increase in the placebo group, approximately 5 min post-exercise. This did not have any apparent affect on plasma choline or choline metabolite levels which remained unchanged under the placebo condition.

Large doses of choline have been reported by Wood and Allison (1982) to cause excessive sweating in non-exercising subjects. Although not directly

measured, it was visibly evident that subjects on the choline treatment were sweating more profusely than subjects on the placebo treatment. None of the other reported studies (Conlay, Wurtman, Blusztajn et al., 1986; Burns, Costill, Fink et al., 1988; Sandage, Sabounjian, White, and Wurtman, 1992; Niels von Allworden, Horn, Kahl, and Feldheim, 1993; Spector, Jackman, Sabounjian et al, 1995) that examined choline supplementation and physical performance noted that excessive sweating occurred. The level of supplementation in these studies was only about one-third the dosage used in this study. One would expect heavy sweating in well-conditioned individuals working at high exercise intensity levels; therefore, other investigators may not have been looking for differences in sweat loss. The physical demands and the many adverse environmental conditions that occur during military training and operations may lead to loss of body water and dehydration, which could jeopardize performance and the mission. Taking doses of choline similar to those used in this study could exacerbate the dehydration condition. Since excessive choline above the dosage used in other performance studies did not show a positive independent effect, it is recommended that any future investigations either use supplements not exceeding a total of 2 g, or study the influence of choline supplementation on sweat loss during exercise.

CHAPTER FOUR

COGNITIVE PERFORMANCE & MOOD STATES

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INTRODUCTION

Most of the literature on the effects of choline on cognition focus on its therapeutic use to slow the effects of age-related neurological diseases such as Alzheimer's or Parkinson's (Lieberman and Abou-Nader, 1986; Wurtman, Hefti, and Melamed, 1981). Treatment of Alzheimer's with choline for less than 2 weeks had little therapeutic effect. However, in a study where choline was administered for up to 6 months, a group of patients did show positive changes in a cognitive test battery (Little, Levy, Chuaqui-Kidd, and Hand, 1985).

In normal subjects given various forms of choline to improve memory or other cognitive functions, results have been conflicting. Improvements in memory, serial learning, and word recognition following intake of choline in various forms (e.g., phosphatidylcholine, choline chloride, and lecithin) have been reported (Ladd, Sommer, Laberge, and Toscano, 1993; Sitaram, Weingartner, and Gillin, 1978; Sorgatz, 1987). Others, however, have shown no effects of choline in cognitive functioning (Davis, Mohs, Tinklenberg et al., 1980; Gillin, Sitaram, Weingartner et al., 1981; Harris, Dysken, Fovall, and Davis, 1983; Mohs, Davis, Tinklenberg, and Hollister, 1980). Given the promise arising from past research, it was hypothesized that providing supplemental choline would aid in maintaining cognitive performance in individuals whose choline levels were depleted by heavy exercise. This study examined these effects at regular time intervals (during and after exercise) using a

military relevant heavy exercise protocol.

Changes in moods have been observed after consumption of various foods in combination with mediating factors such as exercise, time of day, nutritional status, amount of sleep, etc. (Lieberman, Spring, and Garfield, 1986). Unpublished reports have stated that runners and professional basketball players given supplemental choline drinks experience less fatigue late in the competition than when not given choline. Depression has also been observed with high levels of choline in the blood. Based upon this observation, phosphatidylcholine and lithium have been given for those in the manic phase of manic-depressive illness. This treatment has been shown to calm behavior in this condition (Zeisel, 1988).

Research studies examining the effects of supplemental choline on mood states have not been published. This study examined mood differences in those given a supplemental choline drink vs. those given a placebo drink pre- and post-exercise.

METHODS

Matching-to-Sample Test

The matching-to-sample test assessed short-term spatial memory and pattern recognition skills (Appendix E). This test was given on portable notebook computers. Each session lasted for 5 mins. Testing occurred before, during, and after the load carriage march. Testing during the load carriage march occurred while the volunteers continued to exercise on the treadmill. A portable platform for the notebook computer was attached to the front of the treadmill for testing purposes. After testing, it was removed. Measurements were collected at 35, 95, 155, and 215 mins after the start of the march.

The participant first responded by pressing the down arrow key when the word "READY" appeared on the screen. A six by six red and green checkerboard was displayed. The participant would view this red and green sample matrix for as

long as he felt it necessary to determine the red-green pattern. This viewing was timed, and it was followed by a 15 sec delay. Following this delay, two matrices were presented on the screen. The participant had to choose which of these two matrices matched the original sample by pressing the left or right arrow keys. The computer recorded time to choose the matrix and whether the choice was correct or not. A total of 20 trials was administered or testing was halted after 5 mins.

The parameters measured included:

Total Problems Attempted: Number of trials attempted during the 5 min period. A maximum of 20 trials per 5 min test period was possible.

Sample Viewing Time: Amount of time in seconds that the sample was viewed.

Response Time: Amount of time in seconds used to determine which test matrix (left or right) matched the original sample, and needed to press the appropriate arrow key (left or right).

Percentage Correct: Of those problems attempted, the percentage that was answered correctly.

Scanning Visual Vigilance Test (SVT)

This test was given on notebook computers and followed the matching-to-sample test. A complete description of the SVT is available which shows the test to be sensitive to the effects of drugs (caffeine and diphenhydramine) and operational (vigorous continuous exercise) and environmental (cold) stressors (Lieberman, Coffey, and Kobrick, In press). Procedures followed those used previously (Fine, Kobrick, Lieberman et al., 1994), which required the volunteer to detect a faint dot that appeared randomly on the screen for 2 secs. Average presentation of the dot occurred once a minute. Upon detection of the dot the volunteer pressed the space bar on the keyboard as quickly as possible. The computer recorded whether or not

a stimulus was detected and the response time for the detections. Responses made before or after stimulus occurrence were recorded as false alarms. Each session lasted 20 mins with a total of 20 responses recorded. Testing occurred before, during and after the load carriage march. Measurements during the march were collected at 40, 100, 160, and 220 mins after the start of the march.

The parameters measured included:

Correct Hits: Number of correct hits obtained out of a total of 20 possible.

False Alarms: Number of times responded incorrectly; that is, they responded to a stimulus that was not presented.

Response Time: Amount of time in seconds required to see the stimulus and press the space bar on the computer.

Profile of Mood States

The Profile of Mood States (POMS) questionnaire (McNair, Lorr, and Droppleman, 1971) was used to assess subjective mood changes (Appendix F). The POMS is a 65-item adjective rating scale designed to assess six mood scales (tension, depression, anger, vigor, fatigue, and confusion). Each adjective is scored based upon how much the participant felt that feeling from 0 (not at all) to 4 (extremely). The response set of "How You Have Been Feeling During The Past Few Hours" was used. The POMS was administered before and after the load bearing march.

RESULTS

Matching-to-Sample Test

No significant differences in any of the matching-to-sample measures were observed between those consuming placebo vs. those consuming choline (Table

4.1). Additionally, no differences in any of the measures were observed over time (Table 4.2), nor were there any interaction effects (Figure 4.1).

Table 4.1 Means and standard deviations of matching-to-sample measures by supplemental drink consumed.

	PLACEBO	CHOLINE
Problems Attempted	17.2 ± 4.6	17.3 ± 4.6
Viewing Time (sec)	8.8 ± 7.0	9.1 ± 7.0
Response Time (sec)	3.8 ± 1.8	3.8 ± 1.8
Percent Correct	89.6 ± 15.1	88.6 ± 15.1

Scanning Visual Vigilance Test (SVT)

No significant differences in any of the three SVT measures were observed between those consuming placebo vs. those consuming choline (Table 4.3). There were also no differences in any of the measures observed over time (Table 4.4), nor were there any interaction effects Figure 4.2).

Figure 4.1 Interaction effects (Drink X Time) for matching-to-sample measures.

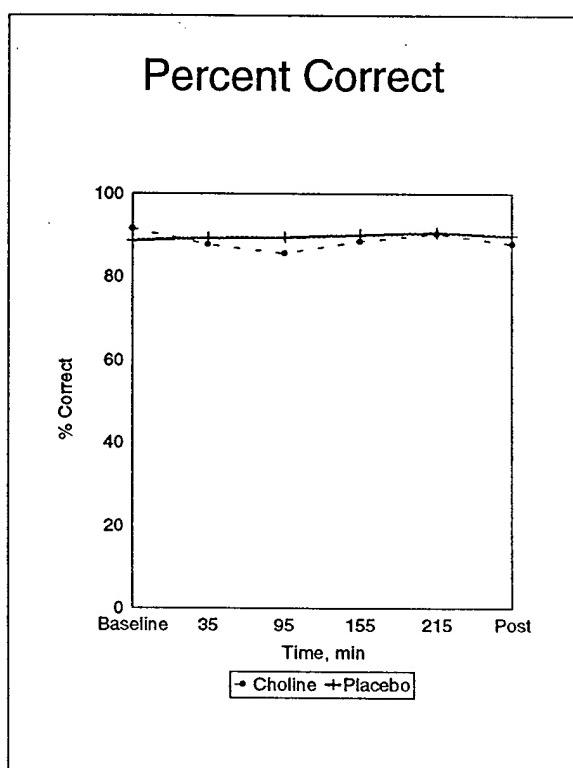
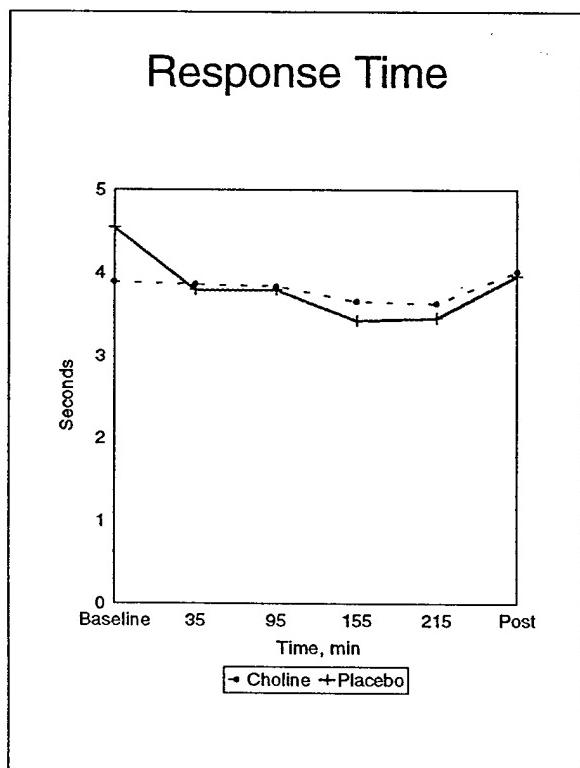
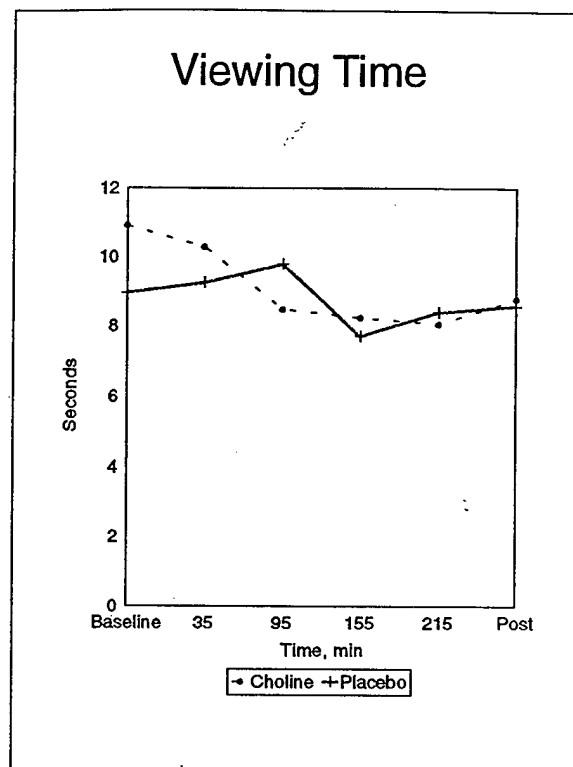
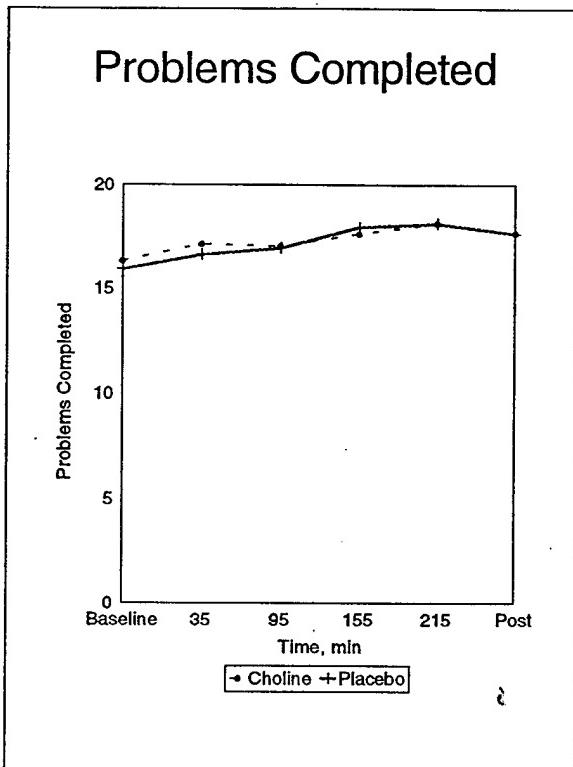


Table 4.2 Means and standard deviations of matching-to-sample measures over time.

	PRE-TEST	35 MIN	95 MIN	155 MIN	215 MIN	POST-TEST
Problems Attempted	16.1 ± 8.1	16.8 ± 8.1	16.9 ± 8.1	17.8 ± 8.1	18.1 ± 8.2	17.6 ± 8.1
Viewing Time (sec)	9.9 ± 12.4	9.8 ± 12.4	9.1 ± 12.4	8.0 ± 12.4	8.2 ± 12.6	8.7 ± 12.4
Response Time (sec)	4.2 ± 3.2	3.8 ± 3.2	3.8 ± 3.2	3.5 ± 3.2	3.5 ± 3.2	4.0 ± 3.2
Percent Correct	90.1 ± 25.8	88.5 ± 25.8	87.5 ± 25.8	89.3 ± 25.8	90.5 ± 26.2	88.8 ± 25.8

Table 4.3 Means and standard deviations of scanning visual vigilance measures by supplemental drink consumed.

	PLACEBO	CHOLINE
Correct Hits	17.7 ± 2.6	17.5 ± 3.2
False Alarms	1.8 ± 2.2	1.7 ± 1.7
Response Time (sec)	1.1 ± 0.2	1.1 ± 0.2

Figure 4.2 Interaction effects (Drink X Time) for scanning visual vigilance measures.

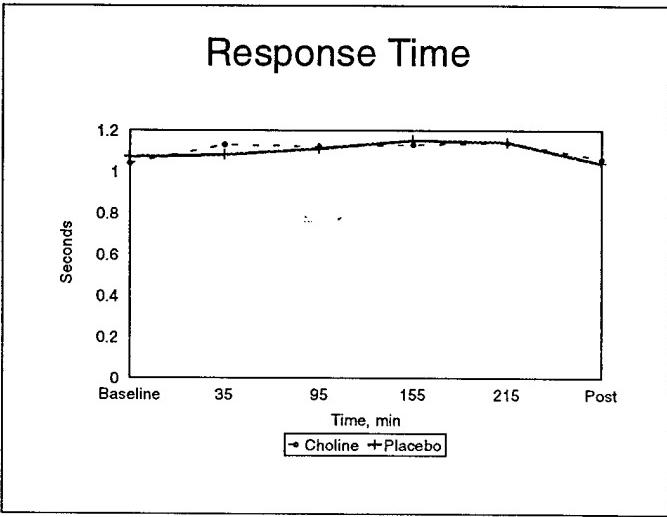
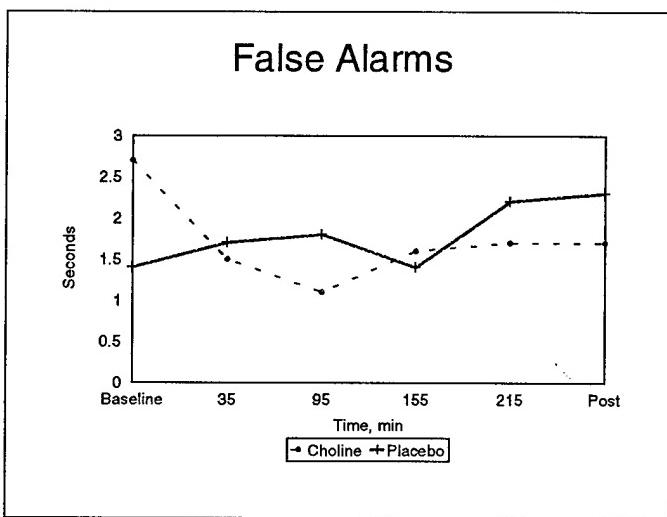
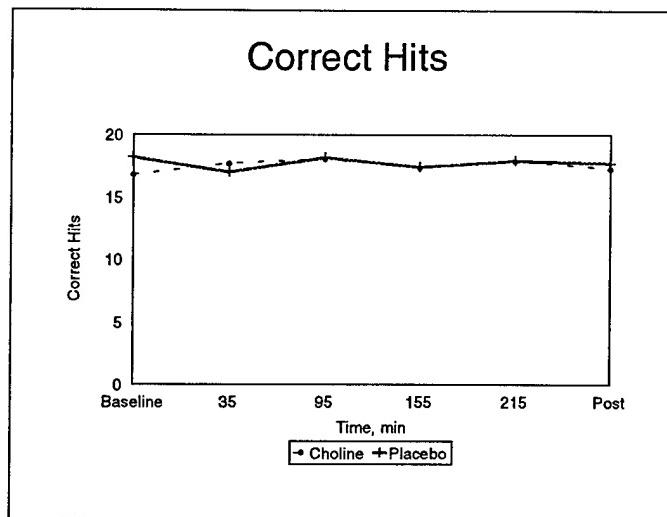


Table 4.4 Means and standard deviations of scanning visual vigilance measures over time.

	PRE-TEST	35 MIN	95 MIN	155 MIN	215 MIN	POST-TEST
Correct Hits	17.5 ± 2.5	17.3 ± 3.3	18.2 ± 2.8	17.4 ± 3.4	17.9 ± 2.7	17.5 ± 2.7
False Alarms	2.1 ± 1.9	1.6 ± 1.9	1.5 ± 1.7	1.5 ± 1.8	1.9 ± 1.9	2.0 ± 2.3
Response Time (sec)	1.06 ± 0.26	1.11 ± 0.22	1.11 ± 0.22	1.14 ± 0.23	1.14 ± 0.22	1.05 ± 0.23

Table 4.5 Means and standard deviations of POMS raw mood scores by choline group.

	PRE-PLACEBO	POST-PLACEBO	PRE-CHOLINE	POST-CHOLINE
Tension	3.7 ± 2.9	3.2 ± 1.6	4.5 ± 2.7	3.4 ± 1.7
Depression	0.8 ± 1.3	0.4 ± 1.3	1.6 ± 2.2	0.6 ± 1.5
Anger	3.6 ± 4.3	3.1 ± 4.0	6.2 ± 5.8	2.4 ± 3.0
Vigor	18.2 ± 5.2	16.1 ± 4.0	17.5 ± 6.2	13.1 ± 6.5
Fatigue	2.1 ± 2.8	7.8 ± 2.9	3.1 ± 3.4	10.1 ± 6.0
Confusion	2.6 ± 1.6	2.7 ± 2.3	2.9 ± 2.1	3.3 ± 2.0

Profile of Mood States

No significant differences in mood states were observed between those consuming placebo vs. choline. The effect of the various exercises did significantly affect some mood states. Reductions in levels of depression $F=9.16(1,13)$, $p<0.01$, anger $F=12.59 (1,13)$, $p<0.005$, and vigor $F=8.34 (1,13)$, $p<0.01$ were observed following exercise testing. Level of fatigue significantly increased after exercise $F=38.02 (1,13)$, $p<0.0001$. Table 4.5 shows various mood changes pre- and post-exercise by choline group.

DISCUSSION

Providing supplemental choline did not improve or maintain cognitive performance as assessed by the matching-to-sample test and the scanning visual vigilance test. The matching-to-sample test is sensitive to cognitive changes as evidenced by past research that saw positive effects with short-term memory tests (Ladd, Sommer, Laberge, and Toscano, 1993; Sitaram, Weingartner, Caine, and Gillin, 1978). Furthermore, an animal version of the matching-to-sample task has been used with cholinergic drugs and has been shown to be sensitive to these compounds (Penetar and McDonough, 1983). No studies using any measures similar to the SVT have been reported previously. An examination of Tables 4.2 and 4.4 show little decrements as a result of the exercise. Therefore, it is possible that these tests were not significantly affected by the exercise, precluding the possible beneficial effects of choline. Furthermore, plasma choline levels were not diminished by the exercise; therefore, decrements arising out of a choline deficiency were not present (Figures 3.3 and 3.4). The supplemented choline group however, did have an increase in plasma choline of 128% over baseline levels (Figures 3.2).

The levels of choline (total of 6 gms) in this study were higher than those given previously (3.75 gms) which elicited positive results on a memory/learning task (Ladd, Sommer, Laberge, and Toscano, 1993). All previous work reporting positive effects of choline on cognitive performance cited slow learners as those most receptive to

realizing improvements associated with choline supplementation (Ladd, Sommer, Laberge, and Toscano, 1993; Sitaram, Weingartner, Caine, and Gillin, 1978; Sorgatz, 1987). Ladd, Sommer, Laberge, and Toscano (1993) suggest that slow learners may have subnormal endogenous levels of choline, which may be elevated by supplementation producing measurable improvements in memory. The subjects within our test were U.S. Army Rangers who are among the elite of Army combat forces. Slow learners probably have been filtered out of our sample population by the selection process negating that as a possible mediating variable. As mentioned above, the exercise did not produce a reduction in plasma choline; therefore, cognitive deficits caused by an exercise-induced reduction in peripheral plasma choline levels likewise were not present.

Mood states were not significantly changed by the administration of choline. As with the matching-to-sample test, since choline deficits were not produced by the exercise, there would be no reason to expect the administration of supplemental choline to improve mood state. While unpublished reports by Interneuron Corporation (Lexington, MA) show vigor was increased and fatigue was reduced in basketball players, it would have been important to know the relationship to plasma choline levels.

The POMS measures of vigor and fatigue showed similar changes in response to carrying a rucksack and an all-out run as was seen previously with similar exercises (Montain, Shippee, and Tharion, 1997). Past research (Folkins, Lynch, and Gardner, 1972; Morgan, Roberts, and Finerman, 1971; Morris and Salmon, 1994) has shown similar mood changes after exercise to the present study; i.e., greater feelings of fatigue, but an overall improved feeling of well-being (lower levels of anger and depression).

To summarize, the results of this study show that providing supplemental choline 1) did not improve cognitive performance or visual vigilance after exercise, or 2) have any effect on mood state. These findings may have been influenced by the lack of an exercise-produced deficit minimizing the need for choline supplementation during strenuous military exercise.

CHAPTER FIVE

MARKSMANSHIP

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INTRODUCTION

Marksmanship accuracy has been shown to be compromised after physically fatiguing military exercises such as carrying a rucksack (Knapik, Staab, Bahrke et al., 1991; Tharion and Moore, 1993), or carrying of patient litters (Tharion, Rice, Sharp, and Marlowe, 1993). Cited causes are muscular fatigue and increased heart rate.

Choline, a nutrient that is present in foods such as eggs, red meats, and fish, has been hypothesized to be important in reducing muscular fatigue. Choline is a precursor to acetylcholine, an important neurotransmitter. Previous research has shown that after exhaustive endurance exercise by elite runners, plasma choline concentrations were lowered (Conlay, Wurtman, Blusztajn, et al., 1986; Wurtman and Lewis, 1991; Sandage, Sabounjian, White, and Wurtman, 1992). Experimental studies with animals suggest that depletion of acetylcholine may contribute to fatigue produced with electrical stimulation (Pagala, Namba, and Grob, 1984). In addition, reductions in serum choline have been found to be associated with a slowing in transmission of the contraction-generating impulse across the muscle (Xia, 1991).

Sandage, Sabounjian, White, and Wurtman (1992) found that supplementing runners with a choline beverage before and during a 20 mile timed run prevented a fall in plasma choline and improved the runners' run times by 5 mins. No research has been published examining the possible beneficial effects of choline on rifle marksmanship. It is hypothesized that shooting after heavy exercise with choline supplements may show smaller decrements in shooting accuracy because the effects of fatigue will be reduced. It is not expected that choline would improve shooting accuracy while rested.

The purpose of this study was to assess the possible use of choline to minimize the fatiguing effects of rucksack carrying on marksmanship accuracy. A second purpose was to assess the effects of typical loads carried in rested, well-trained soldiers. The effects of extremely heavy loads (45 kg) on marksmanship (Tharion and Moore, 1993; Knapik, Staab, Bahrke et al., 1991), and the carrying of average loads (21 kg) while in a caloric deficit (Tharion, Montain, O'Brien et al., 1997) have been shown to affect marksmanship accuracy. What has not been examined are the effects of load carriage on marksmanship under normally encountered conditions. The final objective of this study is to assess the role of physical condition (i.e., measures of strength and oxygen consumption) on the ability to shoot accurately after load carriage.

METHODS

Marksmanship Procedure

Rifle marksmanship was quantified with the Noptel ST-1000 (Noptel KY, Oulu, Finland) laser marksmanship simulator attached to a disabled M-16A1 rifle. The rifle simulator has been shown to be a reliable measure of marksmanship performance in previous studies (Tharion, Montain, O'Brien et al., 1997; Tharion and Moore, 1993; Tharion, Hoyt, Marlowe, and Cyberman, 1992). Marksmanship parameters assessed were distance from center of mass of the target (DCM), shot group tightness (SGT), horizontal shot group tightness (HSGT), vertical shot group tightness (VSGT), horizontal deviation (HDEV), vertical deviation (VDEV), and sighting time (STIME). These measures have been defined previously (Tharion, Hoyt, Marlowe, and Cyberman, 1992).

The simulator consists of a laser transmitter, an optical glass sensitive receiver with an associated paper aiming target, a personal computer, manufacturer supplied software, and a disabled M-16A1 rifle: The laser transmitter emits an invisible continuous 0.55 mm diameter 0.8 μm wavelength beam that allows aiming positions to be monitored and recorded throughout the sighting and shooting process. A vibration sensor in the laser transmitter detects when the weapon is "fired." Shot

location of the laser is recorded via its position on the optical glass laser sensor target. The associated aiming target is a 2.3-cm diameter circular target located 5 m away. This target simulates a 46-cm diameter target at 100 m, which is similar to the standard 49-cm wide, 100-m military silhouette man. Thus, the obtained marksmanship distance and tightness scores would be 20 times greater while shooting at an actual target on a shooting range.

Two days of training with 50 shots taken each day prior to actual testing were provided. Identical procedures were used during testing and training sessions. The pre-exercise marksmanship assessment occurred prior to the administration of the choline drink or any exercise. The post-exercise marksmanship assessment occurred after the 4 hr rucksack carry, the run-to-exhaustion, and the post-exercise blood draw, but before the repeated squat lift. Volunteers shot from the standing offhand unsupported position. They were instructed to shoot as fast and as accurately as possible, since both measures would be scored. Each volunteer fired two sets of five shots. Volunteers were given a ready signal, and after a randomly-varied 1 to 10 sec preparatory interval, were signaled to shoot by the illumination of a red LED stimulus light positioned 8 cm to the left of the target. Sighting time was the time from light illumination to trigger pull. Volunteers were required to hold the rifle barrel below their waist while waiting for the stimulus light to come on.

STATISTICAL ANALYSES

Repeated measures analyses of variance (ANOVA) were used to assess differences 1) before exercise and before consuming the test beverage, 2) after exercise and after consuming the placebo drink, and 3) after exercise and after consuming the choline drink. Prior to the repeated measures ANOVA analyses, two different T-Tests were run on each variable. The first assessed order effect differences (DAY 1 vs. DAY 2) of the post-exercise measures. Initially, the order the volunteers received each beverage was balanced; however, due to dropouts, eight received the placebo beverage first and six received the choline beverage first. No significant order effects were observed for any marksmanship measure ($p>0.05$). The second T-Test was used to examine if there were any differences in the pre-

exercise (rested) pre-beverage (before choline or placebo was consumed) administration for any of the marksmanship measures. No significant differences existed ($p>0.05$). Since no differences existed in the pre-exercise measures over the 2 days, they were averaged for the repeated measures ANOVA. Post-hoc analyses to determine the location of significant differences were done using Tukey's Multiple Comparison Tests. Statistical significance was set at $p\leq 0.05$. Pearson Product-Moment Correlations were run to assess the relationship between $\text{VO}_{2\text{max}}$ and marksmanship, and also between number of squats (using a weight of 45.5 kg) and marksmanship.

RESULTS

The effect of load bearing exercise followed by a timed run-to-exhaustion did not significantly affect marksmanship accuracy ($p>0.05$). Post-exercise sighting time was reduced significantly $F = 9.20$ (2,26), $p\leq 0.001$. Choline did not significantly affect any marksmanship measure. A non-significant trend on the effect of choline on marksmanship accuracy did exist though for SGT. After exercise when taking a placebo supplement, SGT increased an average 12.5% (a decrement), but after exercise and taking the choline beverage, SGT was reduced by 5% (an improvement). Table 5.1 summarizes the effect of exercise and choline for all the marksmanship parameters. The large SGT scores are primarily a reflection of one or more shots missing the target completely. When the target was missed, the distance from the center of the target was recorded as the distance from the edge of the target (115 mm) in the direction of the missed shot. Theoretically, a SGT maximum measurement of $(115)^2 \text{ mm}^2$ could be obtained.

Marksmanship was not correlated ($< \pm .20$) with maximum heart rate or $\text{VO}_{2\text{max}}$. Significant correlations existed between the maximum number of squats using 45.5 kg and post-exercise DCM: $r = -.47$, squats and SGT: $r = -.42$ at $p< 0.10$.

TABLE 5.1 The effects of exercise and choline on marksmanship parameters.

	PRE-EXERCISE	POST-EXERCISE PLACEBO	POST-EXERCISE CHOLINE	SIG. LEVEL
DCM	8.4 ± 1.5	8.7 ± 1.0	8.4 ± 1.7	NS
SGT	154.8 ± 49.6	174.2 ± 58.4	146.4 ± 66.9	NS
HSGT	11.4 ± 2.5	12.7 ± 2.9	10.3 ± 3.3	NS
VSGT	13.3 ± 2.9	13.8 ± 4.0	14.4 ± 4.2	NS
HDEV	-1.5 ± 3.6	-2.7 ± 3.7	-1.0 ± 4.5	NS
VDEV	-1.0 ± 2.0	-0.5 ± 2.9	-0.4 ± 2.9	NS
STIME	9.6 ± 2.9	8.0 ± 2.5 ¹	8.2 ± 2.3 ¹	p<0.01

Values are Means ± S.D.

Degrees of Freedom = (2,26)

¹ Significantly different than Pre-Exercise using Tukey's Post Hoc Tests

Key Abbreviations: DCM = distance from center of mass (mm); SGT = shot group tightness (mm^2); HSGT = horizontal shot group tightness (mm); VSGT = vertical shot group tightness (mm); HDEV = horizontal deviation (mm); VDEV = vertical deviation (mm); STIME = sighting time (sec).

DISCUSSION

The use of choline to help maintain shooting accuracy after fatiguing exercise was not significant. A non-significant trend existed for SGT, which showed that SGT post-exercise after taking choline was maintained compared to pre-exercise values, while after taking the placebo SGT post-exercise showed a 12.5% decrement. The non-significant trend of choline aiding in maintaining shooting performance after heavy exercise is similar to the non-significant positive performance trends found previously in cycling (Spector, Jackman, Sabounjian et al., 1995). The significant performance enhancements found in distance running (Sandage, Sabounjian, White, and Wurtman, 1992) were not found in shooting or cycling and may have been specific to the activity of distance running, although direct comparisons are difficult

because of uncontrolled factors already critiqued by Spector, Jackman, Sabounjian et al. (1995). Alternatively, the effects of the exercise were not significant enough to disrupt shooting accuracy to the point where choline could produce an ergogenic effect.

The 12.5% decrement in SGT is similar but smaller than the 17% decrement both Tharion and Moore (1993), and Knapik, Staab, Bahrke et al. (1991) have reported after carrying loads of 45 kg and 46 kg, respectively. The change from pre- to post-exercise in the present study was not statistically significant. The most obvious reason is the load carried was less and more manageable than that of the previous studies. Another possibility was that the task of using the simulator may not have been fully learned. A greater number of missed targets in the present study resulted in larger DCMs and SGTs than in previous studies using the same methodology (Tharion, Montain, O'Brien et al., 1997, Tharion and Moore, 1993; and Tharion, Hoyt, Marlowe, and Cymerman, 1992). Since the absolute scores are poorer than the previous studies, but no difference was seen pre- to post-exercise, perhaps a learning effect from pre- to post-exercise is responsible for the smaller decrements. Finally, a blood draw which took about five mins with the volunteer sitting on a medical cot may have resulted in some recovery from the exercise, negating the possible adverse affects of an elevated heart rate which has been shown to affect shooting accuracy (Daniels and Landers, 1981; Kruse, Ladefoged, Nielsen et al., 1986; Siitonen, Sonck, and Janne, 1977).

Insignificant correlations between shooting performance and $\dot{V}O_{2\text{max}}$ were seen in this study. Previously, with both male and female elite U.S. biathletes, no significant relationship existed between $\dot{V}O_{2\text{max}}$ and shooting percentage in competition (Rundell and Bacharach, 1995). Significant correlations at $p<0.10$ existed between the number of squats performed with 45.5 kg and DCM and SGT. While not significant at the $p<0.05$ level, the values of $r = -0.42$ and -0.47 are notable considering the lack of training on the simulator and the high intra-subject variability present. The importance of strength to shooting success has been noted previously in female elite biathletes $r = 0.76$. Correlations were not significant with male biathletes (Rundell and Bacharach, 1995). They suggest that once a minimum level of upper body strength is achieved, further improvement does not influence

performance. Since females have lower upper body strength, further improvements in strength could be beneficial to their shooting performance. Although the endurance strength as represented by the squat exercise is likely to be greater in our Ranger population than biathletes, the requirements of having to shoot accurately after rucksack carrying necessitates higher strength requirements. Strength in the legs is important because it reduces body sway, which is important in successful rifle shooting (Niinimaa and McAvoy, 1983). Upper body strength has also been shown to be instrumental in successful shooting (Vercruyssen, Christiana, Muller, and Grose, 1988). The implications for this relationship between squats and shooting are that strength training with high repetitions and a moderate weight is likely to be beneficial in improving rifle shooting accuracy after heavy exercise.

To summarize the results of this study, 1) choline did not improve shooting performance after load carrying exercise, 2) carrying a 21 kg rucksack for 20 km did not significantly degrade shooting accuracy to the level previous marches with heavier loads did, and 3) a positive relationship exists between leg strength assessed via repeated squat exercises and shooting accuracy.

CHAPTER SIX

CONCLUSIONS & RECOMMENDATIONS

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CONCLUSIONS

1. Reduced plasma choline levels have never been documented in endurance events less than 20 miles. The duration and intensity of the load carriage test which amounted to less than 20 miles did not cause a drop in plasma choline under the placebo treatment.
2. Choline did not prove to be an independent factor for improving physical performance. There was no difference between placebo and treatment conditions for any of the physical performance tests.
3. Prolonged load carriage exercise had a positive acute effect on serum HDL levels similar to those reported for marathon runners. Increases in HDL were not significant, but had this been a less trained population with lower fasting levels, the response might have been even greater. Load carriage exercise is an activity that can have a positive effect on plasma lipid levels.
4. The ingestion of 6 grams of free choline under the conditions of prolonged heavy work effected excessive sweating. This potential side effect could have deleterious results under extreme environmental conditions in the field.
5. Choline supplementation was of questionable help in improving shooting performance. Shooting accuracy is dependent upon a minimum amount of lower and upper body strength. Subjects experienced a mean reduction of 50% in lower body

strength as a result of a 20 km load carriage walk. Lower body strength helps to prevent body sway particularly in a standing unsupported firing position. Lower body strength training is important in improving rifle shooting accuracy to compensate for the losses that may occur after performing load carriage training.

6. There is little scientific merit at this time to support the requirement for adding supplemental choline to operational ration components with the expectation it will aid in the improvement of muscle performance. We were unable to cause a choline deficit under the physical conditions of this study.

RECOMMENDATIONS

1. If it is important to keep plasma choline levels from dropping to prevent fatigue and decreased physical performance, it appears lower doses than used in this study would be more appropriate. The dosage used in other studies (1 gram of free choline supplied every 2 to 3 hrs over the course of a continuous exercise bout) should be adequate. This should reduce the likelihood of excess sweating.
2. The primary endurance activity in the military is load carriage walking. It is rare for a unit to cover distances greater than 20 miles in normal training, but it may be important to see if plasma choline is affected by long-distance load carriage equal to or greater than 20 miles in distance.
3. The nutrient choline should be included in the nutrient profile for future analytical analysis of operational ration components to increase the choline nutrient data base.
4. Future testing that examines the acute effects of a potential performance enhancing nutrient should use a military specific exercise task. What works in the athletic or sterile sports model may not work under an equally challenging military relevant field task.

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APPENDIX A

VOLUNTEER AGREEMENT AFFIDAVIT

VOLUNTEER AGREEMENT AFFIDAVIT

This form complies with AR 70-25 and AR 40-38; the proponent agency is OTSG.

PRIVACY ACT OF 1974

Authority: 10 USC 3013, 44 USC 3101, and 10 USC 1071-1087

Principal Purpose: To document voluntary participation in the Clinical Investigation and Research Program. SSN and home address will be used for identification and locating purposes.

Routine Uses: The SSN and home address will be used for identification and locating purposes. Information derived from the study will be used to document the study; implementation of medical programs; adjudication of claims; and for the mandatory reporting of medical conditions as required by law. Information may be furnished to Federal, State, and local agencies.

Disclosure: The furnishing of your SSN and home address is mandatory and necessary to provide identification and to contact you if future information indicates that your health may be adversely affected. Failure to provide the information may preclude your voluntary participation in this investigational study.

PART A — VOLUNTEER AFFIDAVIT

Volunteer Subjects in Approved Department of the Army Research Studies

Volunteers under the provisions of AR 40-38 and AR 70-25 are authorized all necessary medical care for injury or disease which is the proximate result of their participation in such studies.

I, _____, SSN _____, having full capacity to consent and having attained my _____ birthday, do hereby volunteer to participate in "The effect of choline supplementation on physical and mental performance"

(Research study)

under the direction of Harris Lieberman, Ph.D., Natick, MA

conducted at Fort Benning, GA

(Name of Institution)

The implications of my voluntary participation; duration and purpose of the research study; the methods

and means by which it is to be conducted; and the inconveniences and hazards that may reasonably be expected have been explained to me by the USARIEM team.

Contact telephone(s): (508) 651-5128

I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights or study-related injury, I may contact _____

Office of Chief Counsel

at US Army Natick Research, Development, and Engineering Center (508) 651-4322
(Name, Address and Phone Number - include Area Code)

I understand that I may at any time during the course of the study revoke my consent and withdraw from the study without further penalty or loss of benefits; however I may be required (military volunteer) or requested (civilian volunteer) to undergo certain examinations if, in the opinion of the attending physician, such examinations are necessary for my health and well-being. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled.

REVERSE OF NATICK FORM 1487 1 MAR 93

PART B — TO BE COMPLETED BY INVESTIGATOR

INSTRUCTIONS FOR ELEMENTS OF INFORMED CONSENT: (*Provide a detailed explanation in accordance with Appendix C, AR 40-38 or AR 70-25.*)

The nutrient choline which is found naturally in several foods we eat has been considered as a potential performance enhancing compound. Dietary sources of choline include eggs, liver, red meats, fish, peanuts, peanut butter, spinach, cauliflower, iceberg lettuce, and whole wheat bread. The average American consumes about 400 to 900 mg per day of choline. We are asking you to participate in a study to learn about choline and its effect on physical and mental performance. Some preliminary studies indicate it may improve endurance and reduce fatigue. The data from this study will be useful in determining if choline should be used as a dietary supplement in military rations to enhance the performance of combat military personnel.

We will recruit male military volunteers from the 75th Infantry (Ranger) Regiment assigned to Fort Benning, GA. There will be approximately 1 week of training and familiarization of our tests and equipment prior to the actual record testing. Prior to the beginning of the study, you will be medically screened by a USARIEM physician for conditions that would prevent your participation in this study. Each potential subject will be screened, using a medical history and physical examination, for any condition which would prevent safe participation in the study or interfere with data interpretation (prior heat injury, history of foot and joint injuries, back problems or other musculo-skeletal problems, cardiovascular abnormalities, high blood pressure). At this same time, we will collect anthropometric data (height, weight) and ask you to complete a questionnaire on essential background information and lifestyle habits.

For your safety, all electrical equipment will undergo a thorough electrical hazards check.

Treatment: (Twice per day on two separate occasions). One half hour before the first trial with the load carriage walk on the treadmill you will receive a flavored beverage either with (treatment) or without (placebo) choline citrate (neither you nor the research team will know which citrus beverage you received until the completion of the study). You will receive an identical dose at the 2 hr and 50 min break period around the 9 mile point of the load carriage test. This will be repeated during the second trial with the load carriage test but with the other treatment beverage. Once you receive the treatment, you will not be allowed to eat or drink (other than water) until testing is completed for that day. The amount of choline you will receive during the treatment trial is equal to 0.2 ozs (one teaspoon) of pure choline. This amount is about 6 times greater than normal consumption but considered well below the level associated with any side effects, such as intestinal gas, belching or fishy odor to your perspiration. Even these unlikely symptoms are temporary, nontoxic and only slightly undesirable for most people.

This is what we will ask you to do during this study:

Demographics and Lifestyle Habits Questionnaire: This will be given once you agree to take part in this study. This questionnaire asks general questions about you (age, gender, rank). It will also include questions about lifestyle habits, exercise, and dietary supplement practices. This information will help us interpret the results from this study. Completing this questionnaire will take approximately 5 mins.

I do do not *(check one & initial)* consent to the inclusion of this form in my outpatient medical treatment record.

SIGNATURE OF VOLUNTEER	DATE
PERMANENT ADDRESS OF VOLUNTEER	TYPED NAME OF WITNESS
SIGNATURE OF WITNESS	DATE 1 Mar 93

Perceived Exertion: (12 times; 6 times during each load carriage performance). Each soldier's perceived exertion will be assessed at every mile completed after the consumption of placebo or choline treatment beverage beginning after the midexercise break. We will show you a numerical scale with verbal expressions of difficulty to chose your response from. This process will take less than 30 secs each time.

Perceived Treatment and Taste Acceptability Rating: (2 times; 1 time at the completion of each test day). At the completion of each test day, we will ask you to complete a questionnaire asking you 1) about your performance, 2) if you thought you received the placebo or choline treatment, 3) to tell us how much you liked or disliked the beverage supplement you received, and 4) if you had physical symptoms that you attribute to the citrus beverage you received. Completing this questionnaire should take you less than 5 mins.

Blood Samples: (4 times; 2 blood draws per beverage treatment). We will take blood samples at the beginning and at the end of each study treatment to measure the changes in blood levels of choline and other substances as a result of the exercise and drinking the choline beverage. We will take blood samples by needle puncture of a vein in your arm. There is a small risk of a bruise forming, but this will gradually disappear. There is also a possibility that there may be infection and formation of a clot at the puncture site, but the chances that this will occur is slight. This procedure will be performed using a sterile technique by a skilled technician. We will collect a total of about 6.5 tablespoons (0.22 of a pint).

Body Composition: (1 time at the beginning of the study). We will assess your body composition (body fat, muscle, and bone) with an X-ray machine. You will be asked to lie flat on your back on a padded table without moving. This test will take about 10 mins as the scanner moves back and forth above your body. It will cause no pain or discomfort. The total amount of radiation which you will be exposed to from one scan on this machine is about the same amount that you are naturally exposed to in about 3 hrs of sunlight, or about 1/60th of the dose of a chest X-ray.

Treadmill Maximum Oxygen Consumption (VO₂ max) Test: (1 time at the beginning of the study). This test will determine how long you can run and how fast your body can take in and use oxygen. You will run on a motor-driven treadmill 4-5 times for 3 to 5 mins at speeds of 6 to 7 mph. You will get a 5 to 10 min rest period between each run. The runs will be made progressively more difficult by raising the incline of the treadmill until you reach a maximal effort or until you feel you can no longer continue. During the final minute of each run you will breathe into a scuba-type mouthpiece while wearing a noseclip so that we can collect all the air you breathe out. Small paste-on electrodes will be placed on your chest to record your heart rate continuously during the exercise. Accidental bodily injury can result from falling on the treadmill. There will be a spotter during maximal exercise on the treadmill to minimize any possibility of injury. A Medical Monitor (physician) will oversee all of the testing for your health and safety.

Load Carriage Treadmill Test: (2 times; 1 per test day). You will perform a load carriage treadmill test. During this test, you will walk on a motor-driven treadmill for up to 3 hrs and 50 mins at a 3.5 mph pace, up a 3% grade, carrying up to a 65 lb pack. For both trials, the same weight will be carried in the ALICE pack, with additional weight coming from clothing, shoes, and belt (approximately 6 to 8 lbs). A 10 min rest period will be allowed each hour. During each march we will continuously monitor your heart rate and ask you to rate how hard you feel you're working. While walking on the treadmill, in addition to the risks inherent in hard exercise, you may develop blisters or skin chaffing of a temporary nature. There will be an exercise spotter near the treadmill to minimize any possibility of injury. We will monitor physiological functions for your safety and protection. You will not be allowed to continue if any of the following circumstances persist during your time on the treadmill:

(a) Your heart rate exceeds 80% of your maximum heart rate for a period of 5 mins at rest or 90% for a 5 min period during or following exercise.

CONTINUATION PAGE FOR NATICK FORM 1487

Witness's initials	Volunteer's initials

(b) You show evidence of coordination difficulty in walking, development of paleness to your skin, loss of muscle tone, inappropriate respiratory distress, sudden lowering of blood pressure upon changing body positions, enlargement of the pupil of the eyes, or you feel like you are going to faint.

Run to Exhaustion Test: (2 times; 1 per test day). We will measure your body's capacity for endurance activity by its ability to run on a treadmill. This test will be performed after a 10 min rest from completing the load carriage walk. You will exercise by running on a treadmill and gradually increasing exercise intensity by increasing the speed of the treadmill in 2 min steps. At each step we will measure your heart rate. You will continue to exercise to the point of your maximal ability or until you feel you can no longer continue. There is no set amount of time you are expected to run. You can quit when you feel you can no longer keep up with the speed of the treadmill. You may become light-headed when you stop, but this can usually be prevented by continuing to exercise lightly until you recover. You will not have to breathe through any special devices as you did for the VO₂ max test. There will be an exercise spotter near the treadmill to minimize any possibility of injury. We will monitor the same physiological functions as for the other treadmill test for your safety and protection.

NOPTEL Marksmanship Simulator: (4 times; before and after each testing period). Your marksmanship will be measured with a laser marksmanship simulator attached to a disarmed M-16 rifle. The laser transmitter emits an invisible but continuous light that enables the aiming position to be monitored and recorded throughout the sighting and shooting process. This is a timed event. You will be allowed to fire 20 simulated shots (10 shots before load carriage exercise and 10 following the conclusion of the load carriage exercise). Data will be analyzed for time to engage the target and shot accuracy. You will receive practice with the marksmanship simulator before you are tested. DO NOT directly look at the laser nor point the weapon at any other individual, because it may cause eye damage.

Strength/Endurance Test: (3 times; 1 time before the start of the study and once after each treatment period). This is a barbell squat test. The barbell squat exercise requires you to squat with a 100 lb barbell at a continuous and constant visually cued rate. The test is discontinued when you fail to keep up with the established continuous and constant visually cued rate. The score is the number of complete repetitions performed. This test should take no longer than 3 mins. The strength/endurance test (100 lb squat test with free weights) include the potential for straining a muscle and causing temporary soreness or discomfort. There will be a trained spotter for all free weight lifts. This procedure will be administered only by personnel granted privileges through the USARIEM Credentials Committee.

Mental Performance: (4 practice test sessions; 6 test sessions on each load carriage test day (2); Total = 16 test sessions). Your mental performance will be assessed before, during, and after the load carriage test. There will be two tests; both will be taken with a notebook computer. These tests will be explained to you in detail and you will practice them four times during the week before actual testing begins. The visual vigilance test will require you to detect a faint dot on the computer screen and hit the space bar during the 2 secs that it is on the screen. The delayed-match-to-sample test requires you to study a grid pattern of squares on the computer screen and then pick the matching pattern from two grid patterns presented a few seconds later. It is important that you practice the two performance tasks several times on two different days before the study begins, since your mental performance will improve during training. We want you to reach your performance "peak" prior to actual testing. You will perform each task four times during training and will be given feedback on your training performance. You will not be given feedback during the actual study testing.

CONTINUATION PAGE FOR NATICK FORM 1487

Witness's initials	Volunteer's initials
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All psychological testing during the exercise will be paced quickly so that we can assess how well you are able to pay attention and respond. We know from past experience that when volunteers respond verbally to task statements, they may stop answering them when they are uncomfortable, distracted by adverse feelings, or preoccupied. Although you may refuse to answer any questions that you may find intrusive, we ask for your total cooperation and best effort in completing these tasks. Without your involvement, we will lack important information on your reactions and performances for some of the challenging conditions and your response to the choline beverage.

Profile of Mood States Test: (4 times over two days; 2 times during each test session; once before the start of the treadmill walk and once after the finish of the treadmill run-to-exhaustion). Before receiving the beverage and then at the completion of treadmill run which immediately follows the load carriage test, we will ask you to complete this questionnaire about your feelings. These data will tell us about possible mood changes due to choline. It will take you less than 5 mins to complete this computer test.

Food Diary: (2 days prior to start of each testing; 4 days total). We will ask you to complete a 24-hr food record questionnaire for each of the 2 days prior to testing. You will record everything you eat and drink throughout the day, as well as snacks between meals. This information will help us to calculate what nutrients you are currently eating as well as monitor your choline intake from natural dietary sources. Completing this questionnaire will take you approximately 10 mins of your time in the days before testing.

Benefits to You. The benefit of participating in this study is that you will receive detailed information about your body composition, endurance capacity, other physical performance measures, and blood cholesterol levels. You will also have the satisfaction of taking part in a study that will provide important information about how choline affects human physical and mental performance. This nutrient has never been examined under such controlled conditions, and this should tell us if there is something combat soldiers could benefit from through a ration enhancement program. You may request a copy of your personal results, as well as the summary results of the entire study.

Participation in this study is voluntary. If you choose not to take part, or if you choose to withdraw from the study, it will not affect you in any way. You may withdraw from the study at any time with no penalty or adverse action taken against you.

The information you provide, along with the other information we will collect, will be held in strict confidence. The information will be summarized anonymously in all reports that we write about this study, and you and your data will not be identified anywhere in any reports. However, complete confidentiality cannot be promised, particularly to subjects who are military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities. The data we collect may be inspected by the officials of the U.S. Army Medical Research and Materiel Command (USAMRMC).

Before signing this document, make certain that you have read it and fully understand it. If you have any questions concerning this study, please ask us so that you could have better and complete understanding of the study. You may ask questions during the study. We will provide you with a copy of this consent document for your information and your personal record.

The information on the Volunteer Registry Data Sheet (USAMRDC Form 60-R) will be stored at the U.S. Army Medical Research and Materiel Command for future notification purposes should new information become available concerning your participation in this study.

CONTINUATION PAGE FOR NATICK FORM 1487

Witness's initials	Volunteer's initials
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Choline Study Summary

TASK	EXPLANATION
Aerobic Capacity	You will run on a motor driven treadmill until you reach a maximal effort. Small paste-on electrodes will be placed on your chest to record your heart rate. You will also have all the air you breathe out collected at set times.
Body Composition	You will have your body fat analyzed by a "dual-energy X-ray absorptiometry" (DEXA) machine.
2-Day Food Record	You will keep a record of food eaten for 2 days prior to testing
Blood Draw	You will have your blood drawn by phlebotomist just prior to treadmill load carriage test
Treatment	You will get your initial dose of beverage with either 3 g of choline supplement or placebo 30 mins prior to start of treadmill load carriage walk
Mental Performance	You will take a couple of mental test loaded on a computer, 25 mins prior to start of treadmill load carriage walk
<u>Begin Load Carriage</u>	You begin the load carriage walk on the treadmill, it will cover approximately 20 km
Mental Performance	You will repeat the computer-based mental tests at 35 mins and +1 hr 35 mins into the treadmill walk.
Treatment	You will get your second dose of beverage with either choline supplement or placebo at the 2 hr 50 min or 9 mile marker of the walk
Mental Performance	You will repeat the computer-based mental tests at 2 hr 35 mins and 3 hr 35 mins into the treadmill walk
Perceived Exertion	You will verbally rate how tired you are starting every mile after the 7 mile marker
End Load Carriage	You will stop your treadmill load carriage walk after walking for 3 hr 50 mins. You can then remove your rucksack.
Run to Exhaustion	After a 10 min rest after completing the walk, you will be tested for aerobic endurance. You will run on the same treadmill starting at 5 mph; the speed will increase 1 mph every 2 mins until you can no longer keep pace with the speed of the treadmill.
Blood Draw	Identical to the pretest blood draw
Marksmanship	You will fire the M16A1 marksmanship simulator to test speed and accuracy
Strength/Endurance	You will perform as many squats with a 100 lb free weight bar as possible
POMS and Mental Performance	You will complete a written survey form and perform the same mental tests on the notebook computer as done previously.

CONTINUATION PAGE FOR NATICK FORM 1487

<u>Witness's initials</u>	<u>Volunteer's initials</u>
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APPENDIX B

Background Questionnaire

BACKGROUND INFORMATION

Subject Number: _____

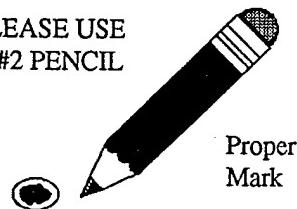
Please answer the following questions for our records. All of the information you provide will be kept confidential. Please use a number two pencil.

1. What is your rank?

E	1	2	3	4	5	6	7	8	9
O									
WO									

2. What was your age on your last birthday? _____ YEARS

PLEASE USE
A #2 PENCIL



3. Which ethnic group do you belong to?

- American Indian/Alaskan Native
- Asian/Pacific Islander
- Black/African
- Hispanic
- White, not of Hispanic origin
- Other (please specify) _____

4. In what part of the country did you live in the longest before age 16? Please fill in one oval.

- New England (ME, NH, VT, MA, CT, RI)
- Middle Atlantic (NJ, NY, PA, DE, MD)
- South Atlantic (DC, VA, WV, NC, SC, GA, FL)
- North Central (OH, IN, IL, MI, WI, MN, IA, MO, ND, SD, NE, KS)
- South Central (KY, TN, AL, MS, AR, LA, OK, TX)
- Mountain (ID, WY, CO, MT, AZ, NM, UT, NV)
- Pacific (WA, OR, CA, AK, HI)
- Other (please specify) _____

5. How long have you been in the Armed Services? _____ YEARS _____ MONTHS

Please Do Not Write in this Box

ID	0	1	2	3	4	5	6	7	8	9
Q2	0	1	2	3	4	5	6	7	8	9

Q3	0	1	2	3	4	5	6	7	8	9
Q4	0	1	2	3	4	5	6	7	8	9

Q5										

6. Please indicate the highest year of school you have completed. Please fill in one oval.

High School Graduate or GED
 College: number of years completed: _____
 Post-Graduate: number of years completed: _____

7. What is your marital status?

- Single, never been married
- Married
- Not Married

8. What is your height? _____ FEET _____ INCHES

9. What is your weight? _____ POUNDS

10. Are you currently trying to:

Lose weight Gain weight Neither

The following set of questions refer to dietary supplements (such as vitamins, Power bars, protein powders, etc.).

11. Have you ever taken any dietary supplements?
If you answered no, please go to question #27.

YES
 NO

12. Are you currently taking supplements?

YES
 NO

13. Estimate how often you take each of the following supplements/
Please fill in one oval next to each supplement below.

ONCE **TWICE OR**
IN A **MORE**
WHILE **TIMES A DAY**

	TIMES A DAY				
Vitamins/Minerals	<input type="radio"/>				
Amino Acids (tablets, powders, drops)	<input checked="" type="radio"/>				
Weight Gain® or Similar Product	<input type="radio"/>				
Herbal Medicines (i.e., ginseng)	<input type="radio"/>				
Carbohydrate/Electrolyte Beverages (i.e., Carbo Fuel, Ultra Fuel)	<input type="radio"/>				
Powdered Protein or Carbohydrate Mixes	<input type="radio"/>				
Appetite Suppressants	<input type="radio"/>				
Choline	<input type="radio"/>				
Carbohydrate Bar (i.e., Power Bar®)	<input type="radio"/>				
Other:	<input type="radio"/>				

	0	1	2	3	4	5	6	7	8	9
College	<input type="text"/>									
	0	1	2	3	4	5	6	7	8	9
Post Grad	<input type="text"/>									
	0	1	2	3	4	5	6	7	8	9
FT	<input type="text"/>									
	0	1	2	3	4	5	6	7	8	9
IN	<input type="text"/>									
	0	1	2	3	4	5	6	7	8	9
WT	<input type="text"/>									

Please Do Not Write in This Box



14. If you take vitamins or minerals, please list them: _____

15. Have you heard of the nutritional supplement called choline? Yes No
If you answered no, please go to question # 21.

Yes No

16. Have you ever taken choline?

If you answered no, please go to question # 21.

17. How often do you take choline?

Never

Once in a while

Once a week

Once a day

Twice or more times day

18. In which form do you get your nutritional intake of choline?

Beverage

Tablet

Diet

19. Do you believe that consuming foods and/or beverages supplemented with choline can affect:
(please mark all that apply)

Overall health

Provide energy for strenuous exercise/training

Improve your mood

Improve your ability to think/concentrate

20. If you heard about the supplement choline from any of the following sources, indicate whether the information you received was unfavorable, neutral, or favorable.

UNFAVORABLE

NEUTRAL

FAVORABLE

Family

Coach or trainer

Friends

Doctor, medic, health care professional

Magazines, TV, or other advertisement

Other source

20a. Received no information

21. Have you heard of the nutritional supplement called lecithin? Yes No
If you answered no, please go to question # 27.

22. Have you ever taken lecithin? Yes No
If you answered no, please go to question # 27.

23. How often do you take lecithin?

Never Once in a while Once a week Once a day Twice or more times day

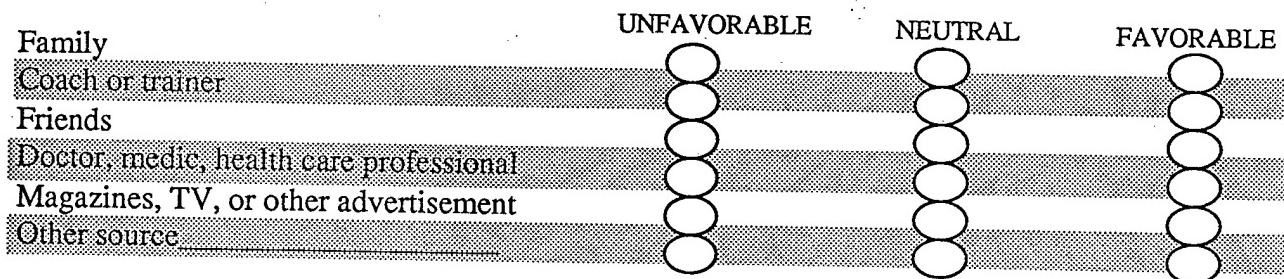
24. In which form do you get your nutritional intake of lecithin?

Beverage Tablet Diet

25. Do you believe that consuming foods and/or beverages supplemented with lecithin can affect:
(please mark all that apply)

- Overall health
 Provide energy for strenuous exercise/training
 Improve your mood
 Improve your ability to think/concentrate

26. If you heard about the supplement lecithin from any of the following sources, indicate whether the information you received was unfavorable, neutral, or favorable.



26a. Received no information

27. How satisfied are with your level of fitness?

Extremely Dissatisfied Moderately Dissatisfied Neither Satisfied Nor Dissatisfied Moderately Satisfied Extremely Satisfied

THANK YOU FOR YOUR PARTICIPATION

APPENDIX C

Daily Record Questionnaire

PLEASE USE
A #2 PENCIL



Daily Record Questionnaire

1. Please rate how you feel right now?

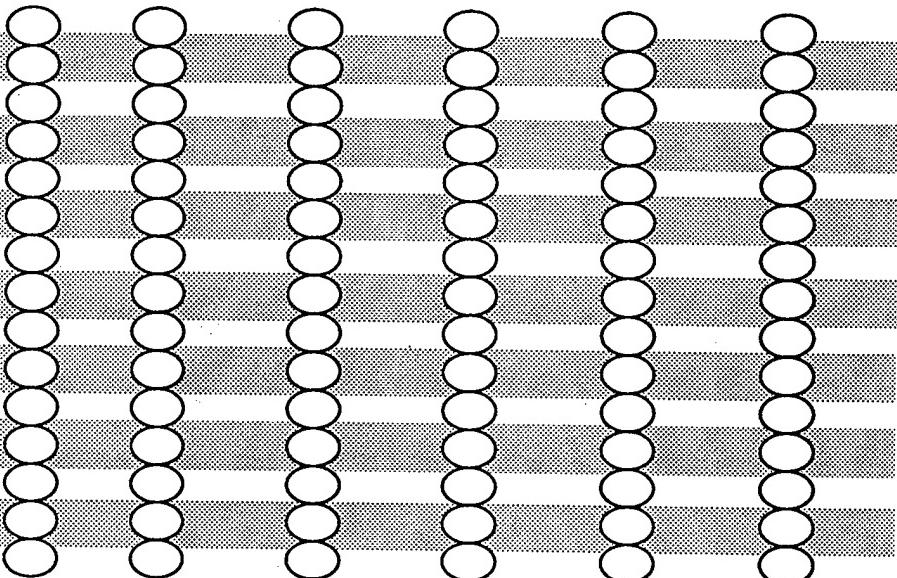
<input type="radio"/> Extremely Bad	<input type="radio"/> Not Very Good	<input type="radio"/> Moderately Bad	<input type="radio"/> Slightly Bad	<input type="radio"/> Neither Good Nor Bad	<input type="radio"/> Slightly Good	<input type="radio"/> Moderately Good	<input type="radio"/> Very Good	<input type="radio"/> Extremely Good
-------------------------------------	-------------------------------------	--------------------------------------	------------------------------------	--	-------------------------------------	---------------------------------------	---------------------------------	--------------------------------------

Proper
Mark

2. Please fill in the oval next to each item to indicate whether you DO or DO NOT have any of the following symptoms.

Not At All Slight Somewhat Moderate Quite A Bit Extremely

- a. I feel tired
- b. I feel energetic
- c. I feel weak
- d. I feel wide awake
- e. My stomach is bloated
- f. I have gas pressure
- g. My coordination is off
- h. I feel dizzy
- i. I lost my appetite
- j. I feel restless
- k. I feel hungry
- l. I feel depressed
- m. I feel happy
- n. I feel anxious
- o. Unusual body odor



3. What was your level of activity yesterday?

LIGHT

1

2

3

4

HEAVY

5

4. Did the beverage affect your performance?

YES

NO

4a. If you answered yes, please write in how it affected you. _____

5. Please rate how much you like or dislike the beverage.

<input type="radio"/> Dislike Extremely	<input type="radio"/> Dislike Very Much	<input type="radio"/> Dislike Moderately	<input type="radio"/> Dislike Slightly	<input type="radio"/> Neither Like Nor Dislike	<input type="radio"/> Like Slightly	<input type="radio"/> Like Moderately	<input type="radio"/> Like Very Much	<input type="radio"/> Like Extremely
--	--	---	---	---	--	--	---	---

6. Which beverage do you think you got today?

- Placebo
- Choline

Why? _____

7. Do you smoke cigarettes?

- Yes
- No

If yes, please write in how many. _____

7a. Did you smoke any cigarettes in the last 24 hours?

- Yes
- No

If yes, please write in how many. _____

THANK YOU FOR YOUR PARTICIPATION

APPENDIX D

Final Questionnaire

Final Questionnaire

PLEASE USE
A #2 PENCIL



1. Think about the two test days you have completed, pick the answer which seems most true.

	Better first session	Better second session	About the same
My physical performance/endurance	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My mental performance	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My mood	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Taste of the drink	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Impact of the drink on:			
a. Physical performance	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
b. Mental performance	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
c. Mood	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

2. Did you notice any differences in the drinks you were given?

Yes No

3. Would you take choline as a nutritional supplement on a daily basis?

Yes No

Please write in why you would or would not. _____

4. What was the number one benefit you received from taking choline this week?

6. If a food item had a high content of choline would you be more apt to consume the item?

Yes

No

Why or why not? _____

7. Do you have any comments about choline? _____

THANK YOU FOR YOUR PARTICIPATION

APPENDIX E

TEST OF COGNITIVE PERFORMANCE

SHORT-TERM MEMORY RECALL TASK

Delayed Match-to-Sample Cognitive Test

The delayed match-to-sample test is a subtest of the Walter Reed Performance Assessment Battery. Performance on this subtest involves both short-term and spatial memory, which are affected by cholinergic manipulations. In this subtest, a sample pattern of 36 red and green grid squares is presented on the computer monitor. The research volunteer studies this pattern and then presses a key on the keyboard. The sample pattern disappears and a blank screen is presented for 4 secs ("delay"). Then two test grid patterns are presented and the volunteer selects the pattern that matches the previous sample pattern. The delayed match-to-sample test consists of 20 trials and takes approximately 8 mins to complete.

APPENDIX F

TEST OF MOOD AND SUBJECTIVE STATES

Profile of Mood States

Self-rated measures of symptoms, mood, and perceived behavioral capabilities will be used to evaluate the volunteers' subjective experiences of the endurance test under treated or placebo conditions. Mood states will be evaluated using the Profile of Mood States (POMS).

The POMS is a questionnaire that assesses Tension-Anxiety; Depression-Dejection; Anger-Hostility; Vigor-Activity; Fatigue-Inertia; and Confusion-Bewilderment. The POMS is a 65-item instrument that requires 5 mins to complete.

PROFILE OF MOOD STATES

SUBJECT NUMBER: _____

DATE: _____

Below is a list of words that describe feelings people have. Please read each one carefully. Then mark ONE square under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST 24 HOURS. Please use a number two pencil to mark the squares.



The numbers refer to these phrases:

NOT AT ALL	A LITTLE	MODERATELY	QUITE A BIT	EXTREMELY
0	1	2	3	4

Friendly	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Unworthy	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Desperate	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Tense	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Spiteful	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Sluggish	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Angry	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Sympathetic	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Rebellious	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Worn out	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Uneasy	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Helpless	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Unhappy	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Restless	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Weary	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Clear-headed	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Unable to concentrate	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Bewildered	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lively	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Fatigued	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Alert	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Confused	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Helpful	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Deceived	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Sorry for things done	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Annoyed	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Furious	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Shaky	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Discouraged	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Efficient	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Listless	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Resentful	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Trusting	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Peeved	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Nervous	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Full of pep	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Considerate	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Lonely	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Bad-tempered	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Sad	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Miserable	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Worthless	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Active	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Muddled	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Forgetful	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
On edge	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Cheerful	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Carefree	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Grouchy	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Bitter	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Terrified	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Blue	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Exhausted	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Guilty	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Energetic	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Anxious	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Vigorous	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Panicky	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Ready to fight	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Uncertain about things	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Hopeless	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Good natured	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Bushed	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Relaxed	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	Gloomy	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	MAKE SURE YOU HAVE ANSWERED EVERY ITEM.	

0 1 2 3 4 5 6 7 8 9

0 1 2 3 4 5 6 7 8 9

Subject
Number

<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
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PLEASE DO NOT
WRITE IN
THIS BOXTest

MONTH

DAY